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**BIOLOGICAL EXCESS PHOSPHORUS REMOVAL
IN
ACTIVATED SLUDGE SYSTEMS**

by

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requirements for the degree of Doctor of
Philosophy at the University of Cape Town.**

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I, MARK CHARLES WENTZEL, hereby declare
that this thesis is my own work and that it has not
been submitted for a degree at another University.

Signed by candidate

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SYNOPSIS

When this investigation was commenced in 1983 two activated sludge systems had been developed in South Africa that accomplish biological excess phosphorus (P) removal, the UCT and modified Bardenpho systems. To predict the P removal in these systems, an empirical model had been developed. In the empirical model, P removal was formulated in terms of some of the system parameters, such as anaerobic mass fraction, available readily biodegradable COD and active mass concentration. Organisms directly implicated in biological excess P removal (polyP organisms) did not feature in the model and the P removal was not linked to any basic biological or biochemical behaviour. Clearly, there was a need for a more fundamentally based model. The objective of this thesis was to develop such a model.

From past work it was accepted that;

- (1) anaerobic/aerobic sequencing in the activated sludge system with wastewater addition to the anaerobic phase is a prerequisite for biological excess P removal,
- (2) release of P in the anaerobic zone is essential for P uptake in the subsequent aerobic zone, to give a net P removal for the system,
- (3) the mass of P removal is related to the mass of readily biodegradable COD in the influent, and
- (4) in the anaerobic zone the polyP organisms sequester substrate and store it as poly- β -hydroxybutyrate (PHB), with polyphosphate (polyP) cleavage and P release, and in the aerobic zone the PHB is utilized for growth and for P uptake and polyP storage.

Accordingly, the investigation commenced by focusing on the P release in the anaerobic zone. From studies on batch, plug flow and series anaerobic reactor systems (operated at 20°C)¹ it was concluded that:

¹All experiments reported in this thesis were conducted at 20°C.

In the anaerobic zone:

- (1) Readily biodegradable COD is converted to short chain fatty acids (SCFA) by non-polyP facultative aerobes.
- (2) The SCFA are sequestered by the polyP organisms at a high rate, with associated P release, so that:
- (3) The rate of sequestration, and P release, is governed by the rate of SCFA production from readily biodegradable COD.
- (4) The rate of P release can be modelled as a first order reaction with respect to the active non-polyP heterotrophic mass and the readily biodegradable COD.

In the aerobic phase:

- (1) P uptake is linearly related to P release.
- (2) Sludge age has a minor effect on the magnitude of P uptake, for a constant P release.

From the overall investigation it was concluded that whereas P release and its kinetics can be modelled without direct consideration of the polyP organisms, P uptake and its kinetics cannot be modelled without explicit incorporation of the polyP organism mass. This conclusion pointed to the need to enquire into the occurrence of the polyP organisms and their characteristics.

Past work had indicated that *Acinetobacter* spp. is the most likely organism mediating biological excess P removal. A study of the prevalence of this organism indicated that it is present in significant concentrations in both anaerobic/anoxic/aerobic systems that exhibit biological excess P removal and completely aerobic systems that do not exhibit biological excess P removal. To obtain more information on this organism, *Acinetobacter* spp. were isolated from the anaerobic/anoxic/aerobic and completely aerobic systems. From an enquiry into their carbon (glucose and acetate) utilization, carbon storage (as PHB) and phosphorus storage (as polyP), it was concluded that imposing conditions conducive to biological excess P removal in a system (by anaerobic/aerobic sequencing) does

not select new *Acinetobacter* strains, but rather stimulates the polyP and PHB accumulating propensities inherent in strains already present. This raised the question as to the biochemical mechanisms whereby polyP and PHB are synthesized and degraded.

In the literature a number of biochemical models had been proposed to explain polyP and PHB metabolism in biological excess P removal. Of these, the model of Comeau, Hall, Hancock and Oldham showed the most promise. This model contained two new key proposals;

- (1) under anaerobic conditions the tricarboxylic acid (Krebs) cycle is operative (a novel proposal) and,
- (2) the proton motive force (pmf) must be maintained (although generally accepted in biochemistry, not considered in the previous models).

However, the model was in concept only and restrictive in what it could explain.

From a study of the biochemical literature, a more general model was developed for the polyP organism *Acinetobacter* spp. incorporating the two proposals of Comeau *et al.*, and quantitatively delineating the various biochemical pathways in the biological excess P removal phenomenon. Two key parameters were identified that appear to regulate the pathways, the ATP/ADP and the NADH/NAD ratios. By having identified these regulatory parameters, the model could be extended to situations other than anaerobic/aerobic biological excess P removal systems. It was found that these regulatory parameters are influenced by substrate concentration, intracellular or extracellular (PHB and, say, acetate respectively) and the presence or absence of external electron acceptor (aerobic, anoxic or anaerobic). In terms of the regulatory parameters (ATP/ADP and NADH/NAD) detailed explanations could be given of the behaviour of *Acinetobacter* spp. over a wide range of conditions.

Having resolved the biochemical aspects, attention could be focussed on the kinetic modelling of P uptake. It was not possible to obtain kinetic information on the polyP organism mass from 'normal' mixed cultures developed in biological excess P removal activated sludge systems for reason that the polyP organisms response was largely swamped by the response of the non-polyP heterotrophic mass. It was concluded that kinetic information on P uptake required cultures in which polyP organisms dominate. One approach to obtain such cultures is to apply pure culture

techniques. However, an analysis of data in the literature on *Acinetobacter* spp. pure cultures indicated that the responses of these organisms in pure cultures were very different from the responses inferred from activated sludge systems. Consequently it was proposed that, instead of pure cultures, enhanced cultures be developed which may be more relevant and supply the necessary information. By enhanced culture is meant the development of a polyP organism culture by selecting a substrate and set of environmental conditions that favour polyP organisms to the extent that these become the dominant primary organisms and dominate the culture response. Growth of competing normal heterotrophic organisms will be curtailed naturally but not deliberately terminated, neither will predation by higher organisms and other interactive effects be positively excluded. Also, a strain (or strains) of polyP organism will be naturally selected that may differ from that artificially selected and grown in pure cultures.

From the biochemical model sets of conditions were identified which would promote the development of polyP organism enhanced cultures. One set is to subject the organism mass to an anaerobic/aerobic sequence with short chain acids fed to the anaerobic phase, conditions present in the Phoredox, modified Bardenpho and UCT systems with acetate as influent. Accordingly, in UCT and modified Bardenpho systems, by starting with 100 percent municipal wastewater as influent, incrementally decreasing the wastewater fraction and increasing the acetate fraction, an enhanced culture of polyP organisms was developed. Samples from the enhanced culture "mixed liquor", using aerobic plate tests and the API identification procedure, indicated that greater than 90 percent of the microorganisms were *Acinetobacter* spp. As the acetate fraction increased, macro- and micro-nutrients and yeast extract addition was necessary to maintain polyP organism growth.

Following these procedures, four enhanced cultures were developed; one using a UCT system at 10 days sludge age and three using modified Bardenpho systems at 20, 10 and 7,5 days sludge ages.

From experimental observations on the steady state responses of the enhanced culture systems, and on batch tests in which mixed liquors drawn from the systems were subject to a variety of conditions, 12 compounds involved in biological excess P removal and 13 processes acting on these compounds were identified. A mechanistic model then was proposed which provides a macroscopic description of the complex interaction between compounds and processes.

Using the mechanistic model as a basis the process rates were formulated mathematically, so also the stoichiometric relationships between the processes and compounds. These were incorporated into a kinetic model for biological excess P removal in enhanced cultures.

To calibrate the model, the stoichiometric and kinetic constants had to be determined. Each constant was determined either from a test in which the constant is isolated and directly measured (e.g. specific endogenous mass loss rate, COD/VSS ratio), or from a test in which the effect of the constant is dominant (e.g. specific rate of acetate sequestration, polyP organism specific yield), or by "curve fitting" to a range of system and batch operating conditions (e.g. maximum specific growth rate, Monod half saturation coefficient).

The calibrated model was applied to simulate the response of a variety of batch tests, and the UCT and modified Bardenpho enhanced culture systems under steady state flow and load conditions. Excellent correlation between the predicted and simulated responses were obtained. It would appear that an acceptable kinetic model describing the behaviour of enhanced polyP organism cultures had been developed.

What of the future? The model is specific to enhanced cultures of polyP organisms in which the effects of the non-polyP organism mass are virtually totally suppressed. The next step should be to integrate the enhanced culture model with the general activated sludge model developed at the University of Cape Town. Preliminary indications are that construction of such an integrated model should not present undue difficulty.

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ABSTRACT

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A theory describing the kinetics of phosphorus (P) release in the anaerobic reactor is presented in terms of the readily biodegradable COD in the influent, the non-polyP heterotrophic mass, the anaerobic mass fraction and the reactor flow regime. Observed P release conforms well to that predicted over a wide range of wastewater characteristics and process conditions. Experimental observations indicate that the magnitude of biological excess P uptake is linked strongly to the magnitude of P release in the anaerobic reactor.

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ABSTRACT

In this chapter carbon utilization, carbon storage as polyhydroxybutyrate (PHB) and phosphorus storage as polyphosphate (polyP) are investigated in *Acinetobacter* spp. isolated from anaerobic/anoxic/aerobic and from completely aerobic activated sludge systems. Isolates from all systems, when cultured aerobically (in synthetic media), grew on acetate and glucose and exhibited PHB and polyP accumulation. It is concluded that: (1) In completely aerobic activated sludge systems treating municipal wastewater, *Acinetobacter* spp. can grow competitively to form a significant fraction of the sludge mass. (2) Introducing anaerobic/aerobic sequencing in aerobic activated sludge systems does not promote the selection of a specific *Acinetobacter* strain capable of PHB and polyP accumulation, but rather stimulates accumulation in strains already present in the aerobic system.

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IN BIOLOGICAL EXCESS PHOSPHORUS REMOVAL
– A BIOCHEMICAL MODEL**

ABSTRACT

A biochemical model is presented that explains the behaviour of *Acinetobacter* spp. in biological excess phosphorus removal activated sludge systems. The model modifies and extends the previous proposals. Two key parameters are identified in controlling polyP and PHB synthesis and degradation, the ATP/ADP and NADH/NAD ratios. The predicted behaviour appears to be consistent with that observed.

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ABSTRACT

In UCT and modified Bardenpho systems, by starting with 100 percent municipal wastewater as influent, incrementally decreasing the wastewater fraction and increasing the acetate fraction, enhanced cultures of polyphosphate (polyP) organisms are developed. Aerobic cultures, using the API procedure, indicate greater than 90 percent of the microorganisms as *Acinetobacter* spp. As the acetate fraction is increased, macro- and micro-nutrients and yeast extract addition is necessary to maintain polyP organism growth. Of the macro-nutrients, magnesium, potassium and calcium have to be available in adequate concentrations for P uptake - magnesium and potassium form the principal counter-ions for stabilizing the polyP chain and, to a lesser extent, calcium; calcium is probably involved in other functions. Acid addition is necessary in the aerobic zone to maintain the pH near neutrality. At sludge ages of 10 and 20 days with 100 percent acetate feed (500 mgCOD/l) removals of about 60 and 50 mgP/l respectively are attained, P/VSS (mgP/mgVSS) of about 0,38 and VSS/TSS (mgVSS/mgTSS) of 0,46.

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ABSTRACT

In Chapter 6, procedures were reported to develop enhanced cultures of polyphosphate organisms in modified Bardenpho and UCT systems. In this chapter, from experimental observations on the steady state response of the enhanced culture systems and on batch tests in which mixed liquors drawn from these systems are subject to a variety of conditions, the compounds involved in biological excess P removal and the processes acting on these compounds are identified. A mechanistic model is proposed that explains the complex interactions between compounds and processes. Qualitatively the behaviour indicated by this model is in agreement with present understanding of the biochemistry of the biological excess P removal phenomenon.

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ABSTRACT

A mathematical model is presented that describes the kinetics and stoichiometry of biological excess phosphorus (P) removal phenomena. The mathematical model is developed from the conceptual mechanistic model, set out in Chapter 7. In the mathematical model 12 compounds and 13 processes are identified as essential to describe the biological excess P removal phenomena. The process rates and stoichiometry are formulated mathematically and displayed in matrix form for ready visualization and systematic computation. The kinetic and stoichiometric constants in the model are evaluated directly or indirectly from experimental observations. The behaviour predicted by the model conforms closely to that observed experimentally.

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ABSTRACT

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LIST OF SYMBOLS*

<u>Symbol</u>	<u>Description</u>
a	Mixed liquor recycle ratio from aerobic to anoxic reactors
a'	Constant used to determine biological excess P removal from P release and metabolic P requirements
A/O	Anoxic/Oxic system
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
API	Analytical Profile Index
ATP	Adenosine triphosphate
b	Specific endogenous mass loss rate (/d)
b_A	Specific endogenous mass loss rate, autotrophs (/d)
b_G	Specific endogenous mass loss rate, polyP organisms (/d)
b_H	Specific endogenous mass loss rate, heterotrophs (/d)
b_{pp}	Specific rate of anaerobic maintenance polyP cleavage (/d)
CoA	Coenzyme A
COD	Chemical oxygen demand (mgCOD/l)
C_{sp}	Stoichiometric ratio ($\Delta P:\Delta S_{bs}$) (mgP/mgCOD)
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen concentration (mgO/l)
f_{cv}	COD/VSS ratio
f	Fractional content
$f_{Ep,A}$	Fraction of autotrophs that is unbiodegradable particulate residue
$f_{Ep,G}$	Fraction of polyP organisms that is unbiodegradable particulate residue
$f_{Es,G}$	Fraction of polyP organisms that is unbiodegradable soluble residue
$f_{EsG,N}$	Fractional nitrogen content of $f_{Es,G}$ (mgN/mgCOD)
$f_{P,rel}$	Stoichiometric ratio (P release:acetate taken up) in anaerobic sequestration (mgP/mgCOD)
f_{P,rel_1}	— for phase 1 sequestration
f_{P,rel_2}	— for phase 2 sequestration
$f_{P,upt}$	Stoichiometric ratio (P uptake:stored PHB utilized) (mgP/mgCOD)
$f_{S,b}$	Fraction of substrate that is biodegradable
$f_{S,bs}$	Fraction of substrate that is readily biodegradable

* Details of the symbol system are given in Appendix A

$f_{S,up}$	Fraction of substrate that is unbiodegradable particulate
$f_{S,us}$	Fraction of substrate that is unbiodegradable soluble
f_{xa}	Anaerobic mass fraction
$f_{ZBA,N}$	Fraction of autotroph biological (active) mass that is nitrogen (mgN/mgCOD)
$f_{ZBA,P}$	Fraction of autotroph biological (active) mass that is phosphorus (mgP/mgCOD)
$f_{ZBG,N}$	Fraction of polyP organism biological (active) mass that is nitrogen (mgN/mgCOD)
$f_{ZBG,P}$	Fraction of polyP organism biological (active) mass that is phosphorus (mgP/mgCOD)
$f_{ZEA,N}$	Fraction of autotroph endogenous mass that is nitrogen (mgN/mgCOD)
$f_{ZEA,P}$	Fraction of autotroph endogenous mass that is phosphorus (mgP/mgCOD)
$f_{ZEG,N}$	Fraction of polyP organism endogenous mass that is nitrogen (mgN/mgCOD)
$f_{ZEG,P}$	Fraction of polyP organism endogenous mass that is phosphorus (mgP/mgCOD)
F/M	Food to microorganism ratio
FAD	Flavin adenine dinucleotide (oxidized form)
FADH	Flavin adenine dinucleotide (reduced form)
HAc	Undissociated acetic acid
i	Index referring to compounds in matrix
IAWPRC	International Association for Water Pollution Research and Control
j	Index referring to processes in matrix
K	First order rate constant for P release model (mgCOD/mgVASS/d)
K_c	Specific rate for conversion of "complex" readily biodegradable COD to short chain fatty acids (mgCOD/mgCOD active mass/d)
K_{LP}	Switching function half saturation coefficient for soluble P uptake (mgP/l)
K_{NA}	Switching function half saturation coefficient for ammonia uptake (mgN/l)
K_{NO}	Switching function half saturation coefficient for nitrate uptake (mgN/l)
K_{OH}	Switching function half saturation coefficient for oxygen uptake (mgO/l)

K_{sG}	Growth rate half saturation coefficient for polyP organisms (mgCOD/l)
K_{sG_1}	— with no soluble P limit
K_{sG_2}	— with soluble P limit
K_{SSEQ}	Switching function half saturation coefficient for acetate uptake (mgCOD/l)
K_p	Specific rate for acetate uptake by the polyP organisms (mgCOD/mgCOD active mass/d)
K_{p_1}	— first phase sequestration
K_{p_2}	— second phase sequestration
K_{xp}	Switching function half saturation coefficient for polyP utilization (mgP/l)
M^+	General form of cation stabilizing the polyP chain
$MX_{B,H}$	Mass of non-polyP heterotrophs in system (mgVASS)
MX_v	Mass of volatile solids in system (mgVSS)
N	Nitrogen
N	Number of anaerobic reactors of equal volume in series (Chapter 3 only)
n	Reactor number in a series of anaerobic reactors
N_{h3}	Ammonia nitrogen concentration (mgN/l)
$N_{o,bs}$	Biodegradable soluble organic nitrogen concentration (mgN/l)
N_{o3}	Nitrate nitrogen concentration (mgN/l)
$N_{o3,r}$	Nitrate nitrogen concentration in r-recycle (mgN/l)
NAD	Nicotinamide adenine dinucleotide (oxidized form)
$NADH$	Nicotinamide adenine dinucleotide (reduced form)
$NADP$	Nicotinamide adenine dinucleotide phosphate (oxidized form)
$NADPH$	Nicotinamide adenine dinucleotide phosphate (reduced form)
NO_2	Nitrite
NO_3	Nitrate
O	Oxygen concentration (mgO/l)
OUR	Oxygen utilization rate (mgO/l/h)
$PorPO_4$ or P_i	General symbols indicating phosphorus or phosphate concentration (mgP/l)
P_{max}	Maximum potential phosphorus concentration (mgP/l)
P_o	Initial phosphorus concentration (mgP/l)
P_{polyP}	Stored polyphosphate concentration (mgP/l)
P_s	Soluble phosphorus concentration (mgP/l)
P_t	Soluble phosphorus concentration at time = t (mgP/l)

PHB	Poly- β -hydroxybutyrate
polyP	Polyphosphate
PSwitch	Constant changing sequestration from phase 1 to phase 2 (mgP/mgCOD active mass)
Q	Influent flow rate to system (ℓ/d)
q	Flow rate of mixed liquor waste stream (ℓ/d)
Q_r	r-recycle flow rate (ℓ/d)
R	Retention time (d)
R_s	System sludge age (d)
r	Mixed liquor recycle ratio from anoxic to anaerobic reactors
S_b	Biodegradable COD concentration (mgCOD/ ℓ)
S_{bi}	Influent biodegradable COD concentration (mgCOD/ ℓ)
S_{bp}	Slowly biodegradable (particulate) COD concentration (mgCOD/ ℓ)
S_{bpi}	Influent slowly biodegradable (particulate) COD concentration (mgCOD/ ℓ)
S_{bs}	Readily biodegradable COD concentration (mgCOD/ ℓ)
$S_{bs,a}$	Readily biodegradable short chain fatty acid COD concentration (mgCOD/ ℓ)
$S_{bs,c}$	Readily biodegradable "complex" COD concentration (mgCOD/ ℓ)
S_{bsa}	Available readily biodegradable COD concentration in the anaerobic reactor (mgCOD/ ℓ)
S_{bsi}	Influent readily biodegradable COD concentration (mgCOD/ ℓ)
S'_{bsi}	Readily biodegradable COD available for conversion per litre influent (mgCOD/ ℓ)
S_{bsn}	Readily biodegradable COD concentration in the n^{th} reactor in series of anaerobic reactors (mgCOD/ ℓ)
S_{enm}	Enmeshed slowly biodegradable (particulate) COD concentration (mgCOD/ ℓ)
S_{phb}	Stored PHB concentration (mgCOD/ ℓ)
S_{ti}	Total influent COD concentration (mgCOD/ ℓ)
S_{up}	Unbiodegradable particulate COD concentration (mgCOD/ ℓ)
S_{us}	Unbiodegradable soluble COD concentration (mgCOD/ ℓ)
SCFA	Short chain fatty acids
$SD_{\bar{x}}$	Standard deviation of the mean
s	Underflow recycle ratio
TCA	Tricarboxylic acid (Krebs) cycle
TKN	Total kjeldahl nitrogen concentration (mgN/ ℓ)
TSS	Total suspended solids

CHAPTER 1

INTRODUCTION

Since the end of the second world war there has been a dramatic upsurge of interest in the removal of nitrogen (N) and phosphorus (P) from wastewaters. This arose from the recognition, during this period, of the eutrophic effects that these two elements have on aquatic ecosystems. One wastewater treatment system, the activated sludge system, showed promise for biological removal of N, and considerable research effort was expended towards understanding of the nitrification-denitrification phenomena, and developing activated sludge systems that give optimal N removal.

Research showed that denitrification was inextricably linked to carbon (COD) metabolism so that optimization of denitrification required an understanding of the kinetics of the biological processes involved in carbon removal. The outcome of this research was that by 1981 a general activated sludge kinetic model had been developed which simulated very closely the observed dynamic behaviour of nitrification-denitrification activated sludge systems, over a wide range of influent flow and load conditions, for constant and daily cyclic states, in-series reactor systems incorporating aerated and unaerated reactors, over a range of sludge ages, from 2 to 70 days.

With regard to P removal, since the first practical activated sludge system for biological excess P removal was reported in 1976, research into this aspect also had been pursued actively. At the time this investigation commenced (1983), the achievements in this area were not to the same level attained in COD removal, nitrification and denitrification - no kinetic model of biological excess P removal had been developed. However, from a practical point of view, research up to 1983 had attained a considerable measure of success, viz:

- (1) Identification of the prerequisites to accomplish biological excess P removal in activated sludge systems, viz, presence of readily biodegradable COD in the influent; anaerobic/aerobic sequencing with the influent discharging to the anaerobic zone; protection of the anaerobic zone from nitrate entry.
- (2) Development of a number of activated sludge systems that incorporate the prerequisites for biological excess P removal, e.g. modified Bardenpho, UCT,

AO/Phoredox systems.

- (3) Development of an empirical model to provide rough estimates of the concentration of P that could be removed by the system.
- (4) Development of guidelines whereby the most appropriate of the P removal systems could be selected, the selected system could be designed and operated as effectively as the situation allowed.

In the empirical model, P removal was formulated in terms of some of the system parameters, such as anaerobic mass fraction, available readily biodegradable COD and active mass concentration. Organisms directly implicated in biological excess P removal did not feature and the P removal phenomenon was not linked to any basic biological or biochemical behaviour. As a consequence designs based on the model had a measure of reliability only within the range of conditions in which the model had been developed; indeed there was a measure of uncertainty even with designs within this range because the basic mechanisms underlying the behaviour were not understood. Clearly, the need existed for a more fundamentally based model.

Accepting that excess P removal is biologically mediated, and cognizant of the success that had been attained with the establishment of a general kinetic model describing the biologically mediated phenomena of COD removal, nitrification and denitrification in the activated sludge system, the research group at the University of Cape Town set themselves the objective of incorporating biological excess P removal into the general model. This thesis records a contribution towards this research objective.

The specific objective set for this thesis was to develop a kinetic model for biological excess P removal. As the research evolved, it became apparent that the kinetic modelling *per se* could not be attempted effectively without attention being given to two closely associated areas, viz. the microbiology and biochemistry of the organisms mediating biological excess P removal. Research in consequence, was directed into the three areas of microbiology, biochemistry and kinetics. In each of these a number of research tasks were identified:

- (1) Microbiology
 - (i) Elucidation of the interaction between non-polyP heterotrophs

and polyP organisms with regard to the conversion of readily biodegradable COD to short chain fatty acids in the anaerobic reactor of biological excess P removal systems.

- (ii) Study of occurrence and characteristics of *Acinetobacter* spp. (representing a typical polyP organism) in biological excess P removal systems.

(2) Biochemistry

- (i) Enquiry into biochemical aspects of the conversion of readily biodegradable COD to short chain fatty acids.
- (ii) Development of a biochemical model for biological excess P removal.

(3) Kinetics

- (i) Mathematical formulation of the kinetics of the conversion of readily biodegradable COD to short chain fatty acids.
- (ii) Development of enhanced cultures of polyP organisms.
- (iii) Identification of the processes and compounds associated with biological excess P removal in the enhanced cultures.
- (iv) Development of a mechanistic model that provides a macroscopic description of the interactions between processes and compounds.
- (v) Mathematical formulation of the process rates and their stoichiometric interactions with the compounds.
- (vi) Incorporation of the formulations into a kinetic model for enhanced polyP organism cultures, its calibration, and verification by application to UCT and modified Bardenpho systems with acetate as influent substrate.

The main body of this thesis deals in detail with each of the tasks identified above.

In the presentation of the research findings, it should be noted that the investigation extended over a period of 5 years. During this period, as tasks attained completion, the results were published as papers. The papers cover a wide spectrum of interests, each paper constituting a relatively complete entity in itself. These papers form the basis for presentation of the thesis. Each chapter in the main body of the thesis has its counterpart as a paper and, in a large measure, retains the structure of the paper except where this leads to unnecessary duplication in different chapters.

CHAPTER 2

HISTORICAL OVERVIEW

1. INTRODUCTION

The biological excess phosphorus (P) removal phenomenon, insofar as it pertains to P removal in activated sludge systems, was noted first in 1959. In the three decades since, understanding, conceptualization and application of the phenomenon has grown from the initial incidental observations to well structured biochemical and kinetic descriptions that are applied in design and control of major full scale works. The impetus for these developments did not stem from a pure scientific interest, it came almost wholly from the recognition, albeit slowly, in the 1960's, of the pivotal rôle that phosphorus plays in eutrophication of aquatic environs.

The only recent appreciation of the rôle of phosphorus as a "pollution" agent is apparent from the report by Dr G J Stander in '*Scientific Council for Africa South of the Sahara (CSA) Specialist Meeting on Water Treatment*' (1960): 'Reference was also made to the possible effect on algal bloom of phosphates due to increasing use of synthetic detergents. The lack of information on the effects of phosphate on the development of algal bloom was noted'.

This recognition, of the rôle that phosphorus plays in eutrophication, together with the massive increase in phosphorus loads to the aquatic environment since the second world war, gave rise to an urgent need to develop effective counter measures to limit the discharge of phosphorus. One such counter measure is biological excess P removal. Initially the research into this phenomenon was engineering orientated, towards developing systems that would achieve excess P removal without undue attention being given to the basic mechanisms that controlled the phenomenon. Only from about 1983, with the increasing involvement of research workers from other disciplines, was the enquiry orientated towards the microbiological/biochemical nature of the phenomenon, and the basic mechanisms identified and incorporated into biochemical and kinetic models.

This evolution of biological excess P removal from its humble beginnings constitutes a fascinating study of the interactions between research workers of very different backgrounds and disciplines. It is likely that if this diversity of interest had not been present, the problem of biological excess P removal would not have been resolved. A review of the historical development of biological excess P removal has

interest therefore not only from the technical research, but also from a philosophical research point of view.

Three main phases of evolutions or development in biological excess P removal can be identified:

- (1) Discovery and incidental observations (1959-1974)
- (2) Parameter identification (1975-1983)
- (3) Mechanistic model (1979-present).

We intend to deal with each of these phases, highlighting the state of knowledge at the beginning of each phase, the limitations and constraints that inhibited advance and the successes achieved. This account will include the present state of the art, including the research findings presented in this manuscript; the immediacy of the research reported here makes, of course, that it is not possible to assess completely objectively its value in a historical context.

2. PERIOD I: DISCOVERY AND INCIDENTAL OBSERVATIONS (1959-1974)

2.1 Discovery

The removal of phosphorus (P) by activated sludge systems in excess of that required for normal organism metabolism first was noted, independently, by two research groups, Srinath *et al.* (1959) in India and Alarcon (1961) in America. Srinath *et al.* observed that in a plant exhibiting excess P removal, if a batch containing a mixture of sludge and raw influent wastewater was continuously aerated, a significant reduction in P, to near zero concentration, took place. From a series of such batch tests they concluded that the magnitude of P reduction appeared to be dependent on the concentration of sludge in the batch reactor and the mass of sewage added. Alarcon (1961), in similar batch studies to those of Srinath *et al.*, also reported an initial high rate in reduction in P but, if aeration was continued for longer than 6 hours, they noted a slow release of P to the bulk liquid. Alarcon concluded that the magnitude of the P uptake under batch test conditions was linked to the intensity of aeration.

Srinath *et al.* and Alarcon advanced no explanation as to why the sludges from certain plants exhibited excess P uptake behaviour and others not, or whether the removal was a biological or physical/chemical phenomenon.

2.2 P uptake

The first structured investigation into the P uptake phenomenon was by Levin and Shapiro (1965). Conceptually, they went out from the hypothesis that the uptake is biological, mediated by the metabolic pathways normal to aerobic organisms, namely the sequence of the Embden-Meyerhof (glycolytic) pathway and tricarboxylic acid (Krebs) cycle, particularly the latter because P uptake takes place during aerobic conditions. They noted that it had been reported that some fungi, algae and bacteria stored phosphates in granular clusters called volutin (e.g. Winkler, 1953); if P could be stored in this fashion by activated sludge then the carbon/phosphorus (C/P) ratio normal to microorganisms would not apply and excess uptake would find an acceptable explanation. They did not develop the volutin hypothesis further, but Moore *et al.* (1969) verified the existence of these volutin granules in sludges that were removing P in excess.

On the basis of their hypothesis, that the P uptake was biological, Levin and Shapiro studied the behaviour of sludges from full-scale systems exhibiting excess P removal. These systems had short sludge ages and were presumably non-nitrifying. Samples *aerated* with and without wastewater addition both exhibited P uptake, but the magnitude of the P uptake and the rate of uptake were *higher* for samples *with* wastewater addition than for ones without, indicating that carbonaceous energy addition promoted uptake; if aeration was prolonged, P release to the bulk liquid commenced, as previously observed by Alarcon (1961). In batch tests on two underflow recycle mixed liquor samples, in which one was aerated and the other not, the aerated one *took up* P while the unaerated one *released* P. The necessity of aeration for P uptake led Levin and Shapiro to conclude that P uptake was for ATP formation via *oxidative phosphorylation* in the tricarboxylic acid (Krebs) cycle with oxygen as electron acceptor. The observation that P was not taken up under anaerobic conditions, but in fact was released, led them to conclude that the Embden-Meyerhof pathway, with associated *substrate phosphorylation*, which remains operative under anaerobic conditions, was not implicated in the uptake. If the tricarboxylic acid (Krebs) cycle was implicated in uptake then, they reasoned, (1) the oxygen tension in the mixed liquor and (2) poisons that inhibit oxidative phosphorylation, should have an influence on P uptake.

- (1) Oxygen tension: Batch experiments at different aeration rates indeed showed that at low rates the P uptake rate was correspondingly low, as the aeration rate increased so uptake rate improved, but at a diminishing tempo to some plateau, i.e. inadequate aeration adversely affected the P uptake

rate but aeration beyond a certain rate did not lead to further improvement. However, they reasoned that high aeration intensity should strip CO_2 and the consequential rise in pH might have caused calcium phosphate to precipitate giving rise to the observed P uptake. To check this, Levin and Shapiro aerated five batches of mixed liquor in which the pH was controlled to remain at 5, 6, 7, 8 and 9 respectively. At pH 7 and 8 the uptake rate was rapid whereas at pH 9 the uptake rate was significantly *lower*. If removal was due to solid calcium phosphate formation, the rate should have been higher at pH 9 than at pH 8; observing the opposite they concluded that the mechanism was not precipitation.

- (2) Inhibition: Levin and Shapiro selected 2,4 dinitrophenol in order to monitor the effects of inhibition on P uptake. This chemical inhibits oxidative phosphorylation (ATP formation) in that it uncouples the electron transport chain and the F_1/F_0 ATPase protein complex which generates ATP. Substrate phosphorylation remains unaffected by the inhibitor. Batch tests on mixed liquor samples aerated with and without 2,4 dinitrophenol showed that the sample with the inhibitor exhibited little P uptake, whereas samples without the inhibitor exhibited marked P uptake. This directly implicated the biochemical process of oxidative ATP generation in the P uptake. It should be noted that both the tricarboxylic acid (Krebs) cycle and electron transport chain can function in the presence of 2,4 dinitrophenol. However these processes occur without associated ATP generation. Thus, it is the actual generation of ATP in the oxidative metabolism phosphorylation process that appeared to be linked to P uptake.

Levin and Shapiro noted release of P to the bulk solution after long periods of aeration, in line with the observations of Alarcon (1961); they offered no explanation as to the cause for this aerobic P release.

2.3 P release

In 1967 Shapiro and Shapiro *et al.* noted that, in full-scale systems removing P in excess, "... the phosphate taken up during aeration was leaking out of the cells during settling." Consequently, they focussed attention on P release in *anaerobic* batch tests on mixed liquor samples *without* substrate addition, and found that:

- (1) The process of P release under anaerobic conditions could be reversed to a process of P uptake if the batch was subsequently aerated.

- (2) The P released in the earlier stage of the release process came from the acid-extractable fraction of the organism mass; only in the later stage was P released from the nucleic acid fraction. This indicated that the initial P release was not due to cell death and lysis.
- (3) P release occurred on addition of the poisons KCN and HgCl_2 (an observation also reported by Sekikawa *et al.*, 1966 with the poison K_2CrO_4).
- (4) The rate of P release per unit of solids, $(dP/dt)/\text{MLSS}$, was constant.

As to the causes for the P release under anaerobic conditions, Shapiro and co-workers hypothesized that the P release was due either to the absence of oxygen or the attainment of a particular redox potential. From experimental investigation they concluded that it was not the absence of oxygen *per se* that initiated P release but rather the redox potential of the solution - P release appeared to be triggered if the redox potential fell below 150 mv.

The conclusion that P release under anaerobic conditions was triggered when the redox potential fell below some critical value was accepted by many subsequent research workers and influenced research programs to as late as 1985, e.g. Barnard (1974), Siebritz *et al.* (1980), Koch and Oldham (1985). The persistence of this hypothesis can be explained only by the complete inability to link any other parameter to the release. It persisted despite findings to the contrary: Randall *et al.* (1970) observed P release almost immediately the oxygen became zero, up to sixty minutes before any significant change in redox potential took place. Randall *et al.* concluded that "phosphate release in general is not primarily dependent upon the level of redox potential, but rather, is dependent on environmental factors which adversely affect cell metabolism, such as depletion of dissolved oxygen and/or organic substrate." They were of the opinion that "bacteriolysis is a major cause of activated sludge phosphate release."

2.4 Summary - Shapiro group

The research contributions of the Shapiro group, from investigations into P uptake and P release, were highly significant and their findings influenced subsequent research investigations until Fuhs and Chen's contribution in 1975. In summary, the investigations of Shapiro and co-workers on mixed liquor from systems exhibiting excess P removal, established that:

- (1) Excess P removal is a biological phenomena, not a physical/chemical one.
- (2) P uptake is via formation of volutin granules internal to micro-organisms; they did not implicate any specific organism.
- (3) The aeration of mixed liquor promotes uptake of P, non-aeration release of P. Continuous alternating aeration with non-aeration causes associated uptake and release of P, but with declining magnitudes. They were of the opinion that the P release is due to the lowering of the redox potential below some critical value.
- (4) Extended aeration periods lead to a gradual release of P.

Although they noted the reciprocal relationship between P uptake and P release they did not enquire into its significance.

2.5 Phostrip process

Levin (1966) utilized the phenomena of P release under anaerobic conditions and P uptake under aerobic conditions to patent a system for P removal, the 'Phostrip process'.

Levin *et al.* (1972) report details of this system: "The process is based on findings that the aeration of mixed liquor can induce activated sludge microorganisms to take up dissolved phosphorus in excess of the amount required for growth. If the air supply is turned off and the sludge organisms are permitted to consume all of the dissolved oxygen the phosphorus previously taken up is released into the liquid phase. However, when aeration is recommenced, the microorganisms again take up the dissolved phosphorus." The Phostrip process consists of a single aeration tank with clarifier; the underflow of the clarifier passes to an anaerobic 'stripping tank' where the sludge settles and P is released. The 'stripped' sludge is returned to the activated sludge system while the supernatant is dosed chemically in a precipitator tank, to precipitate released P which is settled and wasted. The supernatant is returned to the influent flow.

The Phostrip process was based virtually totally on observed behavioural patterns; it did not call on any mechanistic explanation to underpin the process behaviour. It seems that the anaerobic P release was looked on as a means to obtain a high P

concentration in a small volume. There is no indication that the patent holder saw a connection between the anaerobic P release in the 'stripping' tank and the aerobic P uptake in the aeration tank.

2.6 Full-scale observations

The Shapiro group offered no explanation as to why certain full-scale systems remove P in excess, and others not. Subsequently, during the late 1960's and early 1970's, numerous research workers focussed attention on collecting data from full-scale systems removing P in excess to identify conditions in these systems that appeared to promote the phenomenon. This data tended to support the findings of the Shapiro group in addition to providing new information. All the full-scale plants were of the conventional type, i.e. with long plug flow reactors, graduated aeration from the inlet to outlet. All were operated under very short sludge ages, from $1\frac{1}{2}$ to 6 days. Underflow recycle ratios ranged from 0,25:1 to 0,5:1.

Vacker *et al.* (1967), noted the soluble P concentrations at the front end of the aeration tank tended to be higher than the influent concentration, indicating P release. They offered no comment on this observation but rather directed their attention to preventing P release in the secondary settling tanks through correct aeration strategies, because this P release reduced P removal. Scalf *et al.* (1969) observed similar behavioural patterns and stated that 'characteristically, activated sludge plants that are removing phosphate show the influent *mixed liquor* orthophosphate concentration to be much higher than that due to combination of synthesis components.' Scalf *et al.* concluded that 'The higher concentration [of P] is the result of rapid release of phosphorus from the return sludge solids upon *contact* with primary effluent.' (This observation is significant because it is the first report that P release is *induced* by substrate addition.) They noted that the P release appeared to be related to the low dissolved oxygen (DO) concentration at the head of the plug flow reactor. Investigating this release further in batch tests, they concluded that the P release increased with decreased aeration rate or increased addition of oxygen demanding material (BOD,COD). However, they did not link the P release to the subsequent P uptake.

Witherow (1970) extended the work of Scalf *et al.* He presented a graphical plot summarizing the P concentration profiles observed in a number of excess P removal systems: P release was observed at the head of the system, followed by subsequent P uptake. He verified the observation of Scalf *et al.* that the P

release was induced by mixing return sludge and primary effluent.

Milbury *et al.* (1971) noted that all the systems giving excess P removal were plug flow in nature; they recommended length:width ratios of 25:1 in the design of these reactors. Furthermore, they noted that reverse aeration, i.e. higher intensity aeration at the effluent than at the influent end, produced improved P removal. Step feeding to the reactor was to be avoided; with step feeding Milbury *et al.* observed a rapid loss of the P removal propensity. As step feeding provides a mixing régime approaching that of complete mixing, completely mixed régimes accordingly also were not recommended. A further observation of note was that the supernatant of digested sludge from these plants needed to be treated to remove P prior to return to the head of the works for further treatment; if not done the plants lost their P removal propensity even though release and uptake continued as before. This observation indicated that the sludge has a limited P accumulation propensity.

2.7 Discussion

By the end of 1974 one may summarize the knowledge on biological excess P removal as follows:

- (1) Excess P removal is biologically mediated, but no specific organism, or groups of organisms, are implicated.
- (2) P uptake takes place under aerobic conditions and the uptake is promoted by increasing the aeration to some upper limit.
- (3) There is the hypothesis that P uptake is for ATP formation in oxidative phosphorylation; however, this provides no explanation for P storage in volutin granules.
- (4) P is released under conditions where there is a deficiency or absence of oxygen; the release is increased if substrate is added to the mixed liquor.
- (5) There is the hypothesis that the P release is induced when the redox potential is reduced below some critical level.
- (6) P release and P uptake are reversible.

- (7) Excess P removal is observed only in plug flow systems; step feeding and completely mixed systems reduce excess P removal to zero.
- (8) Reversed aeration in plug flow reactors promotes P removal.

When one reviews the work in this period with the privilege of hindsight it becomes apparent that the definitive factor that held back progress was that *the link between anaerobic P release and aerobic P uptake was not recognized*. It was generally accepted that in plug flow reactors, P is released at the head of the reactor due to a lack of oxygen arising from the high oxygen demand near the influent end, but no special significance was attached to this. However, P release in the settling tank was identified as a matter of concern because it directly reduced the net P removal in the system, and attention rather was focussed on this release and on reducing it through application of correct aeration strategies.

This period is characterized by controversy and confusion, with the result that there was little confidence in biological excess P removal as a potential practical technology. Milbury *et al.* (1971), for example, were of the opinion that 'at this time there is no accepted explanation for the precise mechanism of this observed P removal'. Mulbarger *et al.* (1971) went so far as to state 'specialized activated sludge plant design for high level P removal should be avoided and treated as a bonus when and if it occurs'.

3. PERIOD 2: PARAMETER IDENTIFICATION (1975-1983)

The second period can be characterized as one in which there was an endeavour to establish;

- (1) the causes for excess P removal and,
- (2) the conditions necessary to stimulate it in activated sludge systems.

Although there is overlap between the first and second periods, the work of two research 'groups' can be considered to mark the start of this second period, namely Fuhs and Chen (1975) and Barnard (1974-1976)/Nicholls (1975)¹. These two 'groups' adopted diametrically opposite approaches, Fuhs and Chen a microbiological and Barnard/Nicholls an engineering one. The microbiological

¹From the literature it is not possible to determine the exact contribution of Barnard and Nicholls. Consequently the two are reviewed together.

approach was to study the microorganisms that appeared to mediate excess P removal and attempt to relate characteristics of these organisms to excess P removal observed in the system. The engineering approach was to study P removal in the system and attempt to relate or identify conditions in the system that promote excess P removal.

3.1 Microbiological approach

The microbiological approach was ill-developed in the first period. Shapiro and co-workers recognized and demonstrated the biological nature of excess P removal, but did not implicate any organism or organism group. Two investigators during the first period, however, did come to the conclusion that mixed liquors from systems exhibiting excess P removal appeared to differ from those from systems not exhibiting excess P removal:

Wells (1969) commented "The rapid uptake of phosphates by Rilling Road plant sludge and the almost complete absence of uptake by the East and West plant sludges when treated under identical conditions, indicate a fundamental difference in the nature of the sludge itself over and above plant differences which produced the sludge". Yall *et al.* (1970) noted that the mixed liquor in activated sludge systems removing P in excess, when the sewage fed to the system was supplemented with glucose, exhibited an increased affinity for P uptake and an increase in the fermentative microflora, i.e. a change in the sludge composition.

Fuhs and Chen (1975), influenced mainly by the work of the Shapiro group, accepted that the excess P removal was biological in nature, and therefore must be mediated by microorganisms. They decided to isolate these microorganisms and study their characteristics in an attempt to relate these characteristics to the excess P removal phenomenon.

The most significant findings of Fuhs and Chen are:

- (1) They concluded that excess P removal was mediated by either a single microorganism group or several closely related groups. From a series of isolation tests they concluded that *Acinetobacter* was the principal organism genus mediating the P removal.
- (2) From plate tests they concluded that the *Acinetobacter* spp. had the ability

to store large amounts of P as polyphosphate in the presence of all nutrients required for growth under *aerobic* conditions.

- (3) From plate tests *Acinetobacter* spp. accumulated poly- β -hydroxybutyrate (PHB) in the presence of acetate under *aerobic* conditions.
- (4) In studying the nutritional requirements of one *Acinetobacter* strain they had isolated, they concluded that under *aerobic* conditions this strain could not grow on glucose, or similar compounds, but grew on short chain fatty acids. Because these short chain fatty acids cannot be utilized by facultative organisms under *anaerobic* conditions the implication is that the *Acinetobacter* strains are obligate aerobes.
- (5) In broth cultures of the *Acinetobacter* strain, addition of acetate stimulated P release under *anaerobic* conditions, and P uptake under *aerobic* conditions.
- (6) In broth cultures, addition of carbon dioxide (CO₂) stimulated P release under *anaerobic* conditions.

From these findings Fuhs and Chen concluded that "Anaerobic conditions preceding aerobiosis in sewage treatment (therefore) could well be related to the appearance of *Acinetobacter* spp. The principal function of the *anaerobic* state is to establish a facultative *anaerobic* microflora, During *anaerobiosis* this flora would tend to produce compounds such as ethanol, acetate and succinate, which serve as a carbon source for *Acinetobacter* spp.". By adding acetate to *Acinetobacter* pure cultures under *aerobic* conditions they could stimulate P uptake and concluded, therefore, that the *anaerobic* state takes no part in excess P removal *per se* and can be excluded provided short chain fatty acids are supplied to *Acinetobacter* in the *aerobic* state.

Although Fuhs and Chen observed that P release was stimulated by acetate addition under *anaerobic* conditions, they attached no undue significance to this, and did not link the P release to the subsequent uptake. This probably was because Fuhs and Chen were influenced by the findings of the Shapiro group and the Phostrip system in that they investigated the P release from the standpoint of it being a mechanism to strip the P from the sludge rather than an intrinsic part of the excess P removal: They "... found that the *anaerobic* phase is not itself necessary to induce release of phosphate from the bacteria into the supernatant. The CO₂ accumulation and the

lowering of the pH which result from anaerobiosis may be important factors, but they could be obtained by other measures. In our experiments, low pH, addition of a carbon source, and unidentified diffusible substances from fermented sewage were more effective than anaerobiosis.'

3.2 Engineering approach

Barnard (1975a)², while investigating the nitrification/denitrification response of a system he developed for this purpose, the 4-stage Bardenpho system, noted that the system removed P in excess. (For system configuration, see Fig 2.1). In a later paper Barnard (1975b) found there was a correlation between effluent nitrate and phosphorus removal, the higher the effluent nitrate the lower the P removal. Accordingly, he postulated that a condition giving rise to excess P removal is the elimination of nitrates in the effluent, either by preventing nitrification taking place, or by denitrification of the nitrates when these are formed. In yet a later paper, Barnard (1974) postulated that 'the essential requirement for phosphorus removal in biological systems is that during *some* stage before the final stage of the process, the sludge or mixed liquor must pass through an anaerobic stage, during which phosphates may or may not be released, followed by a well aerated aerobic stage, during which the phosphates will either be taken up by the organisms or be precipitated as a result of the change in the redox potential'.

Barnard must be credited as being the first to stipulate that excess P removal requires, *inter alia*, the presence of an anaerobic state. However, he did not specify the location of this state in the system.

The next important development was the recognition that excess P removal is promoted if the anaerobic state is created at the *head* of the activated sludge system, in a reactor receiving the underflow recycle and the influent flow. There is no clarity in the literature as to whom to attribute this important finding, to Barnard or to Nicholls; historically, Nicholls (1975) first reported it, but conventionally it is attributed to Barnard (1976) first. However, these two investigators were working closely together, so that credit is due to both:

Nicholls (1975), noting the excess P removal obtained with the Bardenpho system concluded, in agreement with Barnard (1974), that it was necessary for

²The literature of Barnard is reviewed in its true chronological order, not in the order the papers were published - due to delays in publication the chronological order is disturbed.

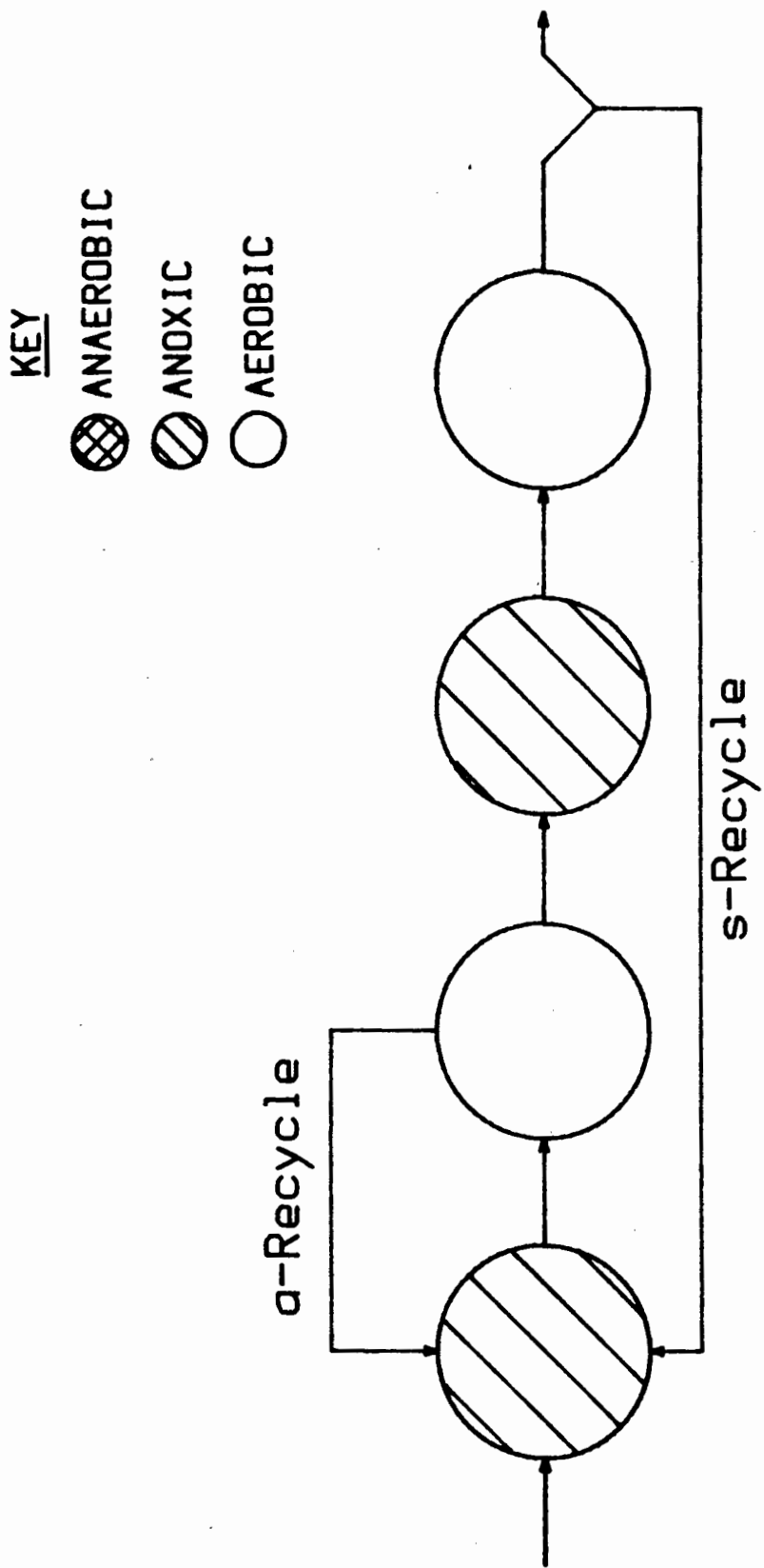


Fig 2.1: Schematic layout of 4-stage Bardenpho activated sludge system.

the sludge to go anaerobic before phosphates were removed. Utilizing this principal, he experimented at full scale with the Alexandra and Olifantsvlei activated sludge systems (Johannesburg, South Africa). He created anaerobic zones in different parts of the system and concluded that "good phosphate removal could be expected in the modified Bardenpho system [actually a 4 stage Bardenpho] where an anaerobic basin is placed *prior* to the activated sludge system".

Barnard (1976), in enlarging on the postulations he developed in 1974, and from a survey of the literature, came to the following general conclusion: "The activated sludge returned from the clarifier or the mixed liquor must pass through an anaerobic phase where oxygen demand exceeds the supply of both oxygen or nitrates at some stage except the final stage before clarification at which point it should be aerated", that "...the degree of anaerobiosis required is such that phosphates need *not* be released but (that) the redox potential at the level at which phosphates are released should be close to the minimum value of the redox potential necessary to ensure good P removal" so that "... whereas it would be difficult to measure the oxidation reduction potential, it would be a simple matter of control to measure the release of phosphates in the anaerobic zone as a means of ensuring the necessary conditions for the removal of phosphates would prevail". To meet these requirements, Barnard proposed the Phoredox system (subsequently termed 5-stage or modified Bardenpho)³. In this system an anaerobic reactor is introduced at the *head* of a 4-stage Bardenpho, and the underflow recycle and influent flow discharged to it. The motivation of Barnard, to include the anaerobic reactor at the head of the system, appears to be that, by discharging the influent flow into this reactor the high oxygen demand would rapidly deplete any oxygen and nitrate entering the reactor, and this would ensure that anaerobic conditions, with the required low redox potential, were achieved.

The work of the Barnard/Nicholls groups is important for the following reasons:

³There is considerable confusion in the names given to this system. During its evolution different names were given to the same system, by Barnard and other investigators. We will follow the following nomenclature - Bardenpho for nitrification/denitrification only; modified Bardenpho for nitrification/denitrification/P removal system in which there is an anaerobic reactor at the head of the system. The modified Bardenpho can include primary and secondary denitrification reactors, i.e. a 5-stage modified Bardenpho (see Fig 2.2), or only the primary denitrification reactor, i.e. a 3-stage modified Bardenpho (see Fig 2.3).

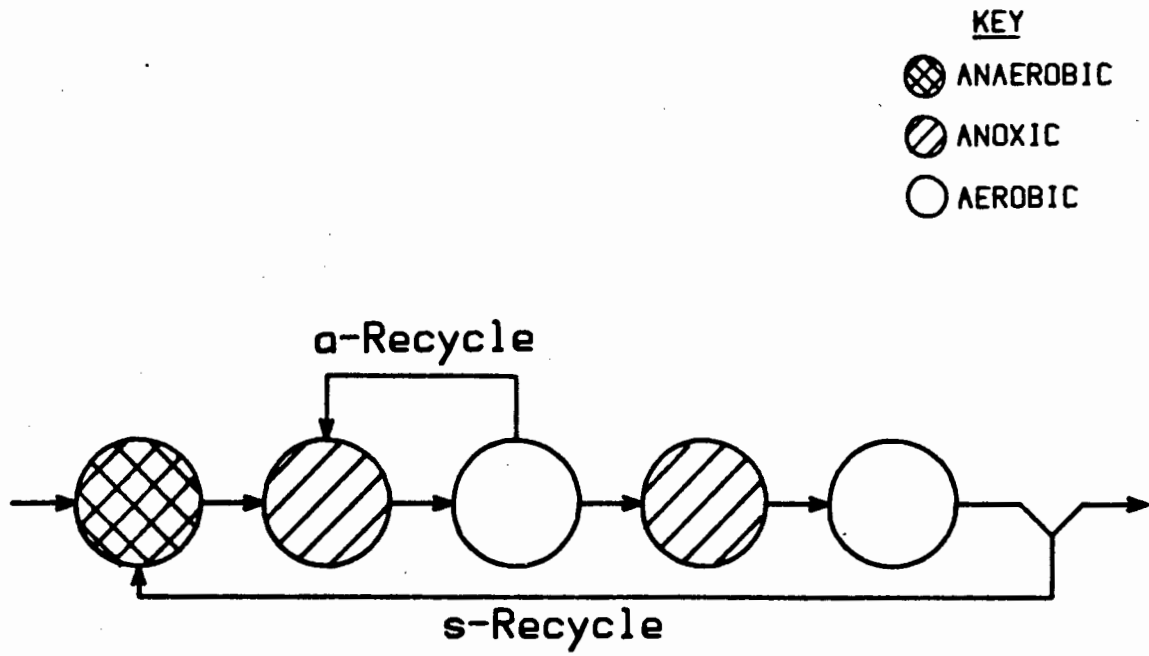


Fig 2.2: Schematic layout of 5-stage modified Bardenpho activated sludge system.

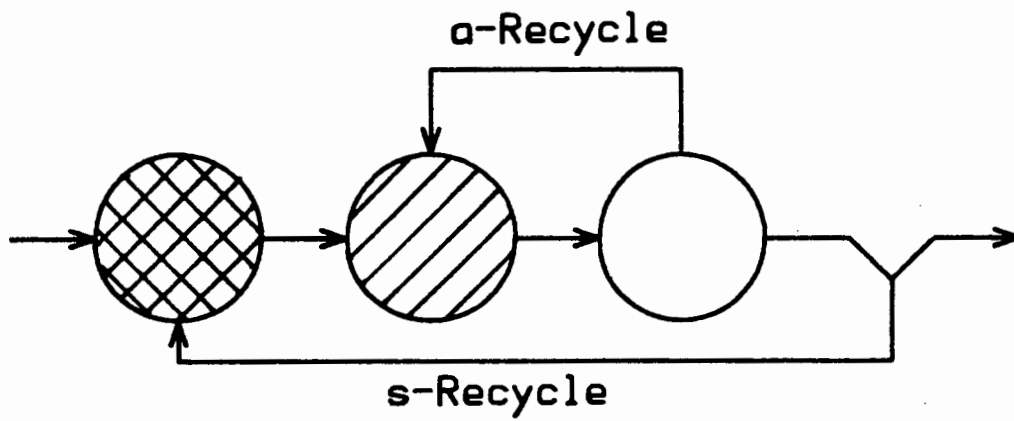


Fig 2.3: Schematic layout of 3-stage modified Bardenpho activated sludge system.

- (1) It linked explicitly the anaerobic state to excess P removal. However, the function of the anaerobic state still was uncertain, and no link was recognized between the P release and P uptake.
- (2) The 5-stage modified Bardenpho system was the first in which specific recommendations, with regard to design and operational requirements, were made to achieve excess P removal. From a research point of view, this formed an important contribution because now it was possible to stimulate the excess P removal, apparently at will, and thereby provide the material for examining the mechanisms involved. From an engineering point of view, a system was available for harnessing the excess P removal phenomenon at full scale.

The work of these two groups, by having evolved a system that appears to incorporate the essential requirements for excess P removal even though these requirements were not explicitly known or understood, stimulated extensive research activity in the field of excess P removal. These subsequent investigators recognized the importance of both the microbiological and engineering approaches, and thus commenced a slow merging of the two approaches. Principally, these investigations were into the function of the anaerobic zone.

3.3 Function of anaerobic zone

Investigating the function of the anaerobic reactor in the 5-stage modified Bardenpho system, McLaren and Wood (1976) concluded that a minimum retention time in this reactor was necessary for P removal and, contrary to the findings of Barnard (1976), noted that there was a strong association between P removal and P release in the anaerobic stage. In contrast, Davelaar *et al.* (1978) concurred with Barnard (1976) in that there did not appear to be any relationship between the amount of P released and the overall percentage removal. This led them to conclude that P release may be a sign of suitable anaerobic conditions rather than an essential intermediate in the process of excess P removal. Furthermore they also found no significant difference in the number of P accumulating bacteria between two systems, one exhibiting excess P removal, the other not. From this they concluded that both sludges had the capacity to induce excess P removal when suitable operating conditions were provided.

Osborn and Nicholls (1978) in an extensive investigation into the excess P removal phenomenon, including literature reviews and experimental results, attempted to

clarify the rôle of the anaerobic zone. From experimental work they concluded that P removal is *directly* associated with changes that take place in the sludge whilst resident in the anaerobic zone; that the function of the anaerobic zone is *not* for the generation of fermentation products for utilization by *Acinetobacter* spp. subsequently in the aerobic zone, as proposed by Fuhs and Chen, for the following reasons:

In an anaerobic/anoxic/aerobic system the fermentation products from the anaerobic zone would *not* be available to *Acinetobacter* in the aerobic zone because these products would be utilized preferentially by the denitrifying organisms in the anoxic zone that preceded the aerobic zone. That is, Osborn and Nicholls had difficulty reconciling the obligate aerobic characteristic of *Acinetobacter* (implied by Fuhs and Chen, 1975, and listed by Bergey, 1974) with the sequencing anaerobic/aerobic requirement for P removal. Furthermore, if *Acinetobacter* is an obligate aerobe, how does it survive anaerobic stress?

Instead, they speculated, a primary requirement for excess P removal is to provide a system which would encourage the selective generation of *facultative anaerobic bacteria*, many of whom may, in addition, have the propensity to store polyP in excess of immediate metabolic requirements. They saw the function of the anaerobic zone in the 5-stage modified Bardenpho system as providing this selective pressure and to activate the generation of polyphosphate kinase in these bacteria. Addition of influent to the anaerobic zone they saw as being necessary only to reduce nitrates in order to induce anaerobic conditions.

Toerien *et al.* (1979) studied the influence of the anaerobic reactor on the bacterial population structure. They concluded that, contrary to the findings of Fuhs and Chen and speculations Osborn and Nicholls and in agreement with the findings of Davelaar *et al.* (1978), the inclusion of an anaerobic zone did not lead to significant selective or enrichment processes in the bacterial populations.

In an endeavour to resolve the question, as to whether specific bacterial populations were selected by the inclusion of an anaerobic zone in excess P removal systems, a number of research workers focussed attention on P accumulation by certain bacteria, in pure culture and activated sludge system studies. Also, they investigated the characteristics of these P accumulating bacteria.

Shoda *et al.* (1980) isolated a bacteria, *Arthrobacter globiformis* PAB-6, capable

of accumulating larger quantities of P. Similarly, Deinema *et al.* (1980) showed that a number of strains of *Acinetobacter* isolated from excess P removal systems are able to accumulate large amounts of P, as well as poly- β -hydroxybutyrate. Further, they demonstrated that these bacteria grow well on lower fatty acids, lower alcohols and lactic acid, and concluded that these compounds may be formed during the anaerobic period by facultative anaerobic bacteria present in the sludge flocs, in agreement with the basic hypothesis of Fuhs and Chen (1975).

Buchan (1981a) studied electron micrographs from several activated sludge systems exhibiting excess P removal and found that all the sludges had microorganisms containing P accumulations in identical intracellular structures. In the electron micrographs he could not locate any extracellular P precipitates so that inorganic precipitation did not appear to be implicated in excess P removal. Also, he found that the P released during anaerobiosis is derived from the intracellular electron-dense bodies. Buchan (1981b), in an extension of the above work, identified the P containing microorganisms to be *Acinetobacter*. From this he concluded that excess biological P removal by activated sludge is dependent on the enrichment of certain *Acinetobacter* in the system.

Summarizing, it would appear that the anaerobic state was necessary to stimulate excess P removal, located at the influent end of the system, but its function was not clear. Questions raised, with regard to the function of the anaerobic reactor, were the following:

- (1) Does it select facultative anaerobic organisms that modify the influent substrate to a form suitable for *Acinetobacter* spp., the *Acinetobacter* spp. mediating the excess P removal? If this is so, how is it possible that the obligate aerobic *Acinetobacter* spp. can grow, maintain themselves and accumulate P in an anaerobic/anoxic/aerobic system?
- (2) Does it select facultative anaerobic organisms that, in addition, accumulate P?

These questions were further complicated by observations that would indicate no significant difference in population structures between sludges removing and not removing P.

3.4 Elimination of the nitrate effect

The research, reported above, would appear to be supportive of the propensity of the 5-stage modified Bardenpho system to stimulate excess P removal. However, a number of research workers found that the system response was unpredictable, either it did not produce any excess P removal, or, removal was sporadic:

Sutton *et al.* (1980) investigated P removal in the 5-stage modified Bardenpho system. In none of their experiments could they obtain excess P removal.

Rabinowitz and Marais in 1977 commenced a study on P removal using the 5-stage modified Bardenpho system treating the unsettled wastewater from the City of Cape Town. The findings of this investigation can be summarized as follows (Rabinowitz and Marais, 1980):

- (1) When the nitrate concentration in the underflow recycle was low, usually P was released in the anaerobic zone and excess P removal was observed. The excess P removal decreased quite disproportionately as the nitrate in the underflow recycle increased.
- (2) However, with different batches of wastewater having the same influent COD within the same concentration of nitrate in the underflow recycle, one wastewater batch may give relatively high P release and high excess P removal whereas the next may give no P release and no excess removal.
- (3) In the main, P removal was disappointing. The system did not give excess P removal over lengthy periods of time, and when P removal was obtained, generally it tended to be low.
- (4) When P removal was poor, or not obtained, increasing the anaerobic reactor size did not lead to an increase in P removal.

Rabinowitz and Marais, in reviewing their unsuccessful endeavours to obtain excess P removal consistently, came to the conclusion that one factor, amongst probably a number, appeared to be of great significance, that is, the nitrate recycled to the anaerobic reactor. (The deleterious effect of the presence of nitrate in the anaerobic reactor subsequently was demonstrated directly by Hascoet and Florentz, 1985). Accordingly, they investigated different system configurations that would shield the anaerobic reactor of any input of nitrate. This led to the development of the

University of Cape Town (UCT) system (see Fig 2.4). In this system the underflow recycle is discharged to the primary anoxic reactor. A further recycle (the r-recycle) draws mixed liquor from the primary anoxic reactor and discharges it to the anaerobic reactor. Mixed liquor also is recycled from the aerobic to the anoxic zone (a-recycle). By manipulation of the a-recycle ratio, the nitrate in the anoxic reactor can be controlled to be zero and thus no nitrate will be recycled to the anaerobic reactor. Consequently the anaerobic state in the reactor can be maintained irrespective of the effluent nitrate concentration. Immediately the UCT system was put into operation, continuous excess P removal was observed, but the removal still fluctuated between one wastewater batch and another, even though with each batch the nitrate recycled to the anaerobic reactor was zero.

Elimination of the confounding effect of recycled nitrate to the anaerobic reactor provided the opportunity to enquire into other factors that influence excess P removal. Here two questions were prominent:

- (1) What causes biological excess P removal to take place?
- (2) How could the P removal be quantified and predicted?

The only answer to (1), up to this stage, had been that the redox potential must be reduced below some critical value, then in an anaerobic/aerobic system P removal will be observed. As regards (2), no answer was forthcoming, although possibly it may be related to the magnitude of depression of the redox potential below the critical value.

The difficulty with redox potential was that it was no simple matter to obtain reliable absolute values in biological systems. To "bypass" this problem, Ekama *et al.* (1979) proposed a surrogate practical measurement, the anaerobic capacity. This they defined as the difference between the mass concentration of nitrate that theoretically could be reduced by the anaerobic reactor if sufficient nitrate was available (denitrification potential) and the actual amount of nitrate discharged to the reactor. They reasoned that, if this capacity was high the redox potential would be low and vice versa.

3.5 Anaerobic capacity

Siebritz *et al.* (1980) accepted that the anaerobic capacity could serve as a substitute parameter for the redox potential and developed this concept further. They

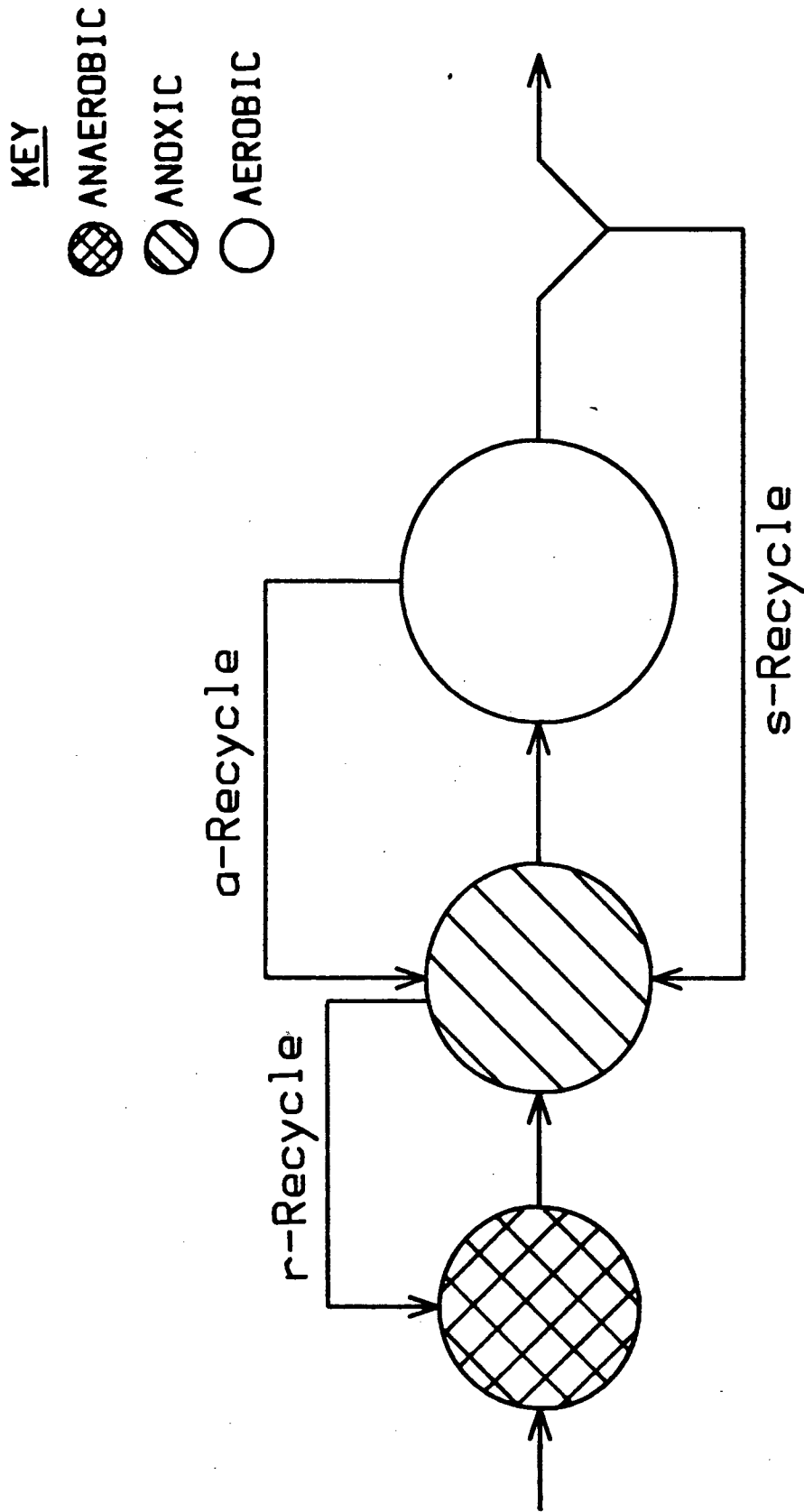


Fig 2.4: Schematic layout of UCT activated sludge system.

speculated that the anaerobic capacity may serve two functions;

- (1) by imposing some critical anaerobic capacity, P release could be induced, and
- (2) the magnitude of the anaerobic capacity greater than the critical may control the magnitude of the P release and the system P removal.

To estimate the anaerobic capacity they applied the work on denitrification developed previously by the Marais group:

Stern and Marais (1974) investigated denitrification in two systems consisting of two-in-series reactor, anoxic/aerobic and aerobic/anoxic respectively. To obtain information on anoxic behaviour they operated the anoxic reactors in a plug-flow regime. They showed that in the anoxic/aerobic system the denitrification took place in two stages, a rapid first stage which persisted for a short period then terminated and a slow second stage which continued over the time each unit of mixed liquor travelled down the plug-flow reactor. In the aerobic/anoxic system only one rate was observed, a very slow one at a rate about half that of the slow second rate in the anoxic/aerobic system.

To explain the two stage denitrification behaviour observed by Stern and Marais in anoxic/aerobic systems, Ekama *et al.* (1979) presented a steady state model for anoxic/aerobic activated sludge systems. In the model they proposed that municipal waste flows contain two biodegradable COD fractions, readily biodegradable and slowly biodegradable respectively. The readily biodegradable COD (S_{bs}) was utilized at a high rate, the slowly biodegradable COD at a rate about one-seventh that of the readily biodegradable COD; the readily biodegradable COD accounted for the initial high denitrification rate and the slowly biodegradable COD for the slower second stage denitrification. With regard to the much lower rate of denitrification in the aerobic/anoxic system, they proposed this to be due to utilization of substrate generated by endogenous mass loss. These concepts were incorporated in a general activated sludge by Dold *et al.* (1980) and van Haandel *et al.* (1981). The extended model of van Haandel *et al.* incorporated the steady state model of Ekama *et al.* (1979) and served as the basic model to analyze nitrification and denitrification effects over a wide range of system configurations and sludge ages.

Siebritz *et al.* applied the theory on denitrification described above in an experimental investigation into the validity of the anaerobic capacity hypothesis in the 5-stage modified Bardenpho and UCT systems. Their results indicated that, if the anaerobic capacity exceeded about 10 mgNO₃-N/l in the anaerobic reactor, P release was observed and there was an associated P removal. Also, as the anaerobic capacity increased above 10 mgNO₃-N/l so the P removal increased, but the correlation was not high. To test the generality of these findings they checked the hypothesis against experimental results on a two reactor anoxic/aerobic system. This system was operated at 20 days sludge age with an underflow recycle of 1:1. The anoxic mass fraction was increased in steps, eventually up to 70 percent. At this anoxic mass fraction the anaerobic capacity was 34 mgNO₃-N/l. Despite this large anaerobic capacity no P release was observed in the first (anoxic) reactor, and only minimal excess P removal was obtained. In contrast, in a UCT system operated in parallel on the same sewage with an anaerobic mass fraction of 7.5 percent giving an anaerobic capacity of 12 mgNO₃-N/l, there was P release in the anaerobic reactor and significant excess P removal.

The only evident difference between the two systems was that in the UCT system an appreciable concentration of readily biodegradable COD would be present in the anaerobic reactor whereas in the anoxic/aerobic system the nitrate recycled to the first (i.e. anoxic) reactor would have completely utilized the readily biodegradable COD, that is, the anaerobic capacity would be generated totally from the utilization of slowly biodegradable COD. This difference in behaviour led them to postulate that it is *the presence of readily biodegradable COD in the anaerobic reactor that induces P release and excess removal.*

3.6 Readily biodegradable COD hypothesis

Extensive research into the validity of the readily biodegradable COD hypothesis over a year by Siebritz *et al.* (1983) established that P release appears to be induced if the readily biodegradable COD in the anaerobic reactor, S_{bsa} , exceeds about 25 mg/l, the release and excess removal increasing as $(S_{bsa}-25)$ increases. That is, the P removal was linearly related to the readily biodegradable COD concentration in the anaerobic reactor. This opened the way for enquiry into other factors affecting the release and excess removal, and quantization of the excess removal. They came to the conclusion that excess P removal depended on three factors;

- (1) $(S_{bsa}-25)$,

3.7 Discussion

The achievements of the second period can be summarized as follows:

From Microbiological studies:

- (1) Excess P removal is mediated by a single organism or a group of closely related organisms. *Acinetobacter* appears to be the principal organism genus mediating the excess P removal.
- (2) *Acinetobacter* spp. can accumulate large quantities of P in intracellular granules called volutin in the presence of a balanced nutrient medium.
- (3) *Acinetobacter* spp. can accumulate PHB.
- (4) Anaerobic/aerobic conditions appear to promote the growth of P accumulating organisms. This aspect is particularly intriguing because *Acinetobacter* are reputed to be obligate aerobes, yet these organisms apparently find advantage for growth by passing through the anaerobic phase with substrate present (as indicated by their abundance in P removal plants, Buchan 1981b). This behaviour is the more remarkable when it is noted that acetate promotes the growth of these organisms in the system even though this substrate is completely unavailable as an energy source to aerobes in the anaerobic zone, being one of the end products of the Embden-Meyerhof pathway.

From Engineering studies:

- (1) Anaerobic/aerobic sequencing of the mixed liquor stimulates excess P removal. The anaerobic state must be induced at the head of the system, as applied in the UCT and 5-stage modified Bardenpho systems.
- (2) Recycling of nitrate or oxygen has a deleterious effect on excess P removal.
- (3) P release to the bulk liquid takes place under anaerobic conditions if readily biodegradable COD is present in the bulk liquid.
- (4) P uptake takes place if P release has been obtained in the anaerobic reactor and nitrate or oxygen is present.

- (5) P release, uptake and excess removal are related to the concentration of readily biodegradable COD in the influent – addition of acetate or glucose to the influent is reflected in improved P removal.

The major contribution of the second period is that two systems had been developed that accomplish biological excess P removal. The basis for these system developments rested on the identification of parameters, physical (in the system configuration), operational (in the recycle magnitudes and aeration or non-aeration of reactors), chemical (in the readily biodegradable COD) and kinetic (in the aerobic and anoxic actions of heterotrophs, nitrifiers and denitrifiers). P release and P uptake did not enter directly into the development, P removal was in a sense 'tagged on', formulated empirically in terms of some of the parameters, e.g. the anaerobic mass fraction, readily biodegradable COD and active mass production; the organisms directly implicated in biological excess P removal were not incorporated as a parameter. Essentially, the approach was virtually totally heuristic - excess P removal behaviour was not explained in terms of, or linked to, any basic biological or biochemical phenomena.

With the work of Siebritz *et al.* the empirical engineering approach appeared to have reached the limit of its productive life. Henceforth the microbiological/biochemical approach would come to dominate further developments in understanding of the biological excess P removal phenomenon.

4. PERIOD 3 : MECHANISTIC APPROACH (1979-present)

The third period is characterized by a major effort to;

- (1) develop mechanistic models of the biological excess P removal phenomena, to delineate the biochemical process and associated products and,
- (2) integrate these in a kinetic model.

Even though the empirical parametric approach in the second period had produced a practical procedure for design, the design rules that had evolved were restricted to the type of plants on which the research data was obtained, within the range of operating conditions imposed. The empirical model, for example, could not be applied in the design of short sludge age systems such as the Phoredox/AO systems. It had become increasingly evident that, until a satisfactory biochemically based model for biological excess P removal was developed, the full potential of the

phenomenon could not be achieved, except by trial and error.

In tracing the development of a biochemical model for biological excess P removal it is worthwhile to review briefly, from a general microbiological viewpoint, the antecedence of the phenomenon of P accumulation by microorganisms.

Harold (1966) in an authoritative survey of the state of the art reports that phosphate accumulation in polyphosphate granules (volutin) is widespread among microorganisms and has been observed amongst others in bacteria, yeasts, fungi and photosynthetic algae. Phosphorus plays such a crucial role in biology that it must have been associated with life from the beginning. There is the suggestion that polyphosphates (polyP) and/or pyrophosphates predate adenosine triphosphates (ATP) as an energy carrier, that polyphosphate is a metabolic fossil which in time has lost its original function to assume new ones 'which still elude us' (Harold 1966). From an evolution point of view if one considers the rapid adaptability and the frequency of mutation in microorganisms, any such new functions would have developed only if these accrued some advantage to the organism in its competitive struggle for survival, otherwise their presence would have diminished with time. It seems therefore that if one could identify the function(s) of polyP accumulations it would go a long way to elucidating their biochemical behaviour.

With regard to function, Harold states that there is no clarity as to whether the polyP accumulations serve the function of phosphate storage or energy storage. He tended towards the phosphate storage hypothesis on the basis that the accumulation of polyP will suffice for several doublings of the cell mass should subsequent phosphate starvation occur — starvation of P is not unlikely, in nature P availability generally is low; also 'polyP could constitute an accessible reserve for synthesis of messenger RNA, ribosomes and metabolic intermediates to facilitate the *initiation* of growth'. Furthermore, he was of the opinion that storage of polyP 'would minimize disturbance of the osmotic equilibrium and of the concentrations of the critical intermediates, Pi, and adenine nucleotides.'

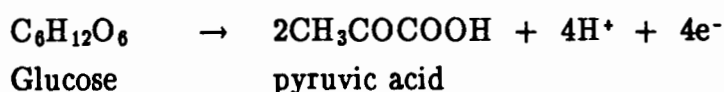
With regard to the cause for polyP storage, Harold noted that temporary limitations of phosphorus or other nutrients (e.g. sulphur and nitrogen), could result in accumulation of polyP in certain bacteria, called 'overplus' and 'luxury uptake' respectively. This would indicate that P accumulation is a response to a stress condition in which the propensity for P accumulation confers

an advantage on the organism in a way not evident, the stress condition being induced by nutrient limitation. However, accumulation in activated sludge systems was found to be not due to nutrient limitation: Fuhs and Chen (1975) in their investigation of P accumulation as polyP in *Acinetobacter* demonstrated the ability of this organism to accumulate polyP in the presence of a complete growth medium, i.e. no nutrient was limiting. From this it was evident that nutrient limitation was not the stress condition that caused polyP accumulation in biological excess P removal systems.

4.1 Nicholls, Hall, Osborn model

Taking due cognisance of the past work, set out above, the Johannesburg group (Hall *et al.*, 1978 and Nicholls and Osborn, 1979) presented the first comprehensive mechanistic model of biological excess P removal. They approached the subject from the standpoint that the polyP phenomena was a response by the organism to the imposition of a stress, not a nutrient limitation but an *anaerobic* stress, and that the polyP pools assist the organisms in *surviving* the stressed state.

From a study of the literature and their own work, they noted a consistent association between polyP and PHB accumulation. Recognizing the importance of these two polymers in the excess P removal phenomenon, they found numerous references to these polymers in the microbiological and biochemical literature, unfortunately areas of enquiry that found few readers in the wastewater treatment field, probably the reason why knowledge available on the polymers had not been applied to excess P removal at an earlier date. They were of the opinion that these high energy polymers were stored for survival purposes under the set of imposed anaerobic/aerobic conditions. With regard to the function of the polymers, they concurred with Harold that polyP served as a phosphorus storage mechanism; as for PHB, they proposed that it "... plays an important rôle in maintaining the life cycle of aerobic and denitrifying organisms during their passage through the anaerobic zone". They proposed the following biochemical model to explain biological excess P removal; "under normal aerobic conditions sewage (as typified by glucose in the following equation) is oxidized in a series of steps to pyruvate, which results in the production of 4 hydrogen atoms and the liberation of 4 electrons

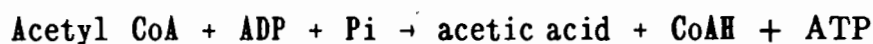


These hydrogen ions and electrons are then passed through the Krebs (Citric acid)

cycle and finally meet up with oxygen from either the air or from nitrate, and are eliminated from the system as water.

*Under anaerobic conditions aerobic bacteria can metabolize substrate up to the formation of acetyl coenzyme A, and will die at this point if no alternative route is available to relieve the system of the accumulated hydrogen ions and associated electrons. Some bacteria have the ability to absorb these hydrogen ions and convert them into water insoluble poly- β -hydroxybutyrate where they can be, as it were, temporarily stored until aerobic conditions are restored. The poly- β -hydroxybutyrate reverts back to acetyl coenzyme A which then passes the liberated hydrogen out of the system as water via the aerobic Krebs cycle.

*Energy requirements for obligate aerobes finding themselves in an anaerobic environment can be supplied as indicated in the following equation:



where the ATP formed is used to support life-sustaining reactions. The phosphorus requirements are probably preferentially supplied from the polyphosphate pool.

*Many aerobic bacteria have the ability to form poly- β -hydroxybutyrate and the concept of this compound acting as a balancing facility for hydrogen ions, or as an electron sink, as it is now more commonly called, is probably the basis of survival of many bacteria when exposed to temporary conditions of anaerobiasis."

The biochemical model of the Johannesburg group can be summarized as follows:

In the anaerobic phase:

- (1) Carbohydrate (glucose) is taken up by polyP organisms and metabolized, via the glycolytic (Embden-Meyerhof) pathway, to acetyl-CoA and electrons and protons.
- (2) The acetyl CoA has two functions; first, to act as an electron and proton sink by its reduction to PHB and second, to act as an energy source for ATP synthesis via deactivation to acetic acid.

- (3) The rôle of polyP is to serve as a source of P in the formation of ATP in (2) above.
- (4) The ATP generated in (2) above is utilized for cell maintenance via hydrolysis to ADP + P_i; such P_i is released to the medium.

In the aerobic phase:

- (1) The stored PHB is utilized as a carbon and energy source for cell function and an energy source for polyP generation and storage.

In support of their model the Johannesburg group set out quantitative biochemical pathways.

From the description of the Johannesburg group's model, it is evident that the orientation is towards survival of the anaerobic stress. This is achieved by;

- (1) creation of a balancing facility for hydrogen ions and electrons, generated in the Embden-Meyerhof pathway, by PHB formation and,
- (2) utilization of acetyl CoA as an energy source for ATP production, the P requirements for this production being supplied by the polyP pool.

The Johannesburg model is important for a number of reasons:

- (1) It focusses attention on the biochemical nature of the phenomenon.
- (2) It links the P uptake in the aerobic zone to PHB storage in the anaerobic zone.
- (3) It shows that PHB storage in the anaerobic zone requires the presence of substrate.

However the model is oriented towards explaining *survival* of the polyP organisms in the anaerobic zone, that is, the model does not explain the apparent advantage the anaerobic zone provides these organisms as indicated by their proliferation in anaerobic/aerobic systems.

4.2 Rensink model

The next conceptualized model of biological excess P removal was proposed by Rensink (1981). This model also is orientated towards survival. Rensink accepted the work of Fuhs and Chen (1975) that *Acinetobacter* spp. is the principal organism implicated in excess P removal and that these organisms only utilize short chain fatty acids as substrate. Also, he accepted Fuhs and Chen's hypothesis that the purpose of the anaerobic state is to enable an assemblage of facultative organisms to develop which produced short chain fatty acids to serve as substrate for the *Acinetobacter* spp. Furthermore he presupposed that *Acinetobacter* spp. is a slow growing obligate aerobe and, in a purely aerobic environment, would not be competitive for substrate with other obligate aerobes; however, by having the propensity for PHB storage in the anaerobic zone, competition for substrate in the aerobic zone is removed enabling the *Acinetobacter* spp. to survive in the anaerobic/aerobic cycle.

Rensink demonstrated the disappearance of soluble COD and release of P^3 under anaerobic conditions, and the uptake of P under subsequent aerobic conditions. Rensink also found close association between P release and uptake. With these ideas as background he proposed the following conceptual model:

In the anaerobic phase:

- (1) Short chain fatty acids (for example acetate) serve as substrate for *Acinetobacter*.
- (2) The short chain fatty acids are stored as PHB, such a process requiring energy (ATP).
- (3) The energy (ATP) requirements in (2) above are supplied by breakdown (hydrolysis) of polyP to free phosphate, the phosphate being released to the bulk solution.

In the aerobic phase:

- (1) Rensink follows the proposals of the Johannesburg group.

Rensink did not propose any biochemical pathways for PHB or polyP synthesis and breakdown.

From the description of Rensink's conceptual model it is evident that he approached the problem from the same standpoint as the Johannesburg group, i.e. survival. However, his model differs in that:

- (1) The reason for substrate storage, as PHB, in the anaerobic zone is to remove the polyP organisms from competition for substrate subsequently in the aerobic zone.
- (2) In the anaerobic zone polyP is used to supply the energy requirements for PHB synthesis.
- (3) Because polyP breakdown (with P release) in the anaerobic zone is for PHB synthesis, and the PHB thus formed subsequently is utilized for polyP synthesis (with P uptake) in the aerobic zone, *aerobic P uptake is implicitly linked to anaerobic P release.*
- (4) Short chain fatty acids are the substrate source for PHB synthesis.

By approaching the problem from a survival standpoint, the model of Rensink, as noted for the Johannesburg group's model, cannot explain the proliferation of polyP accumulating bacteria in anaerobic/aerobic sequencing systems.

The findings of Fukase et al. (1982) provide support for the anaerobic behaviour hypothesized in Rensink's model: They investigated P release and substrate sequestration in the anaerobic zone and found a constant relationship between acetate (or glucose) that disappeared and P released under anaerobic conditions. From this, and other data, they inferred that polyphosphate is hydrolyzed under anaerobic conditions and the energy produced utilized to take up organic matter and store it.

4.3 Marais, Loewenthal, Siebritz model

Marais *et al.* (1983) developed the next conceptual model for biological excess P removal. In agreement with the Johannesburg and Rensink models, they accepted that polyP is broken down and PHB formed under anaerobic conditions, and that polyP is formed and PHB utilized under aerobic conditions. However, in contrast to these two previous models, they approached the problem from a different standpoint: They hypothesized that the incorporation of anaerobic conditions in activated sludge systems confers an *advantage* on the polyP organisms causing them to proliferate.

Their results indicated that only the readily biodegradable COD fraction of the influent was implicated in the P removal phenomenon. Accordingly they proposed that 'PolyP accumulation serves as an energy reservoir, to sustain the organism during the anaerobic stressed state, but principally to gain a positive *advantage* over non-P accumulating organisms by partitioning of readily biodegradable COD (in the lower fatty acid form) in the anaerobic state for its exclusive use subsequently in the aerobic state.'

Marais *et al.* concurred with Rensink that polyP serves as an energy source for PHB storage. Accordingly, they investigated theoretical biochemical pathways for PHB synthesis from the readily biodegradable COD. They suggested that readily biodegradable COD could consist of either a glucose-like material or a short chain fatty acid (e.g. acetate).

With *acetate* as substrate they found that it was not possible, both stoichiometrically and bioenergetically, to put forward pathways for PHB synthesis by invoking established pathways - conversion of acetate as far as acetoacetate only appeared to be feasible. The problem was that with acetate as substrate no source of protons and electrons, to reduce acetoacetate to PHB, was available. They were unable, therefore, to find an explanation for PHB storage under anaerobic conditions with acetate substrate.

With *glucose* as substrate under anaerobic conditions they identified two situations, either (1) the polyP organisms cannot utilize glucose anaerobically, or (2) they can.

- (1) PolyP organisms cannot utilize glucose: In this situation Marais *et al.* proposed that the glucose is converted to acetate by the non-polyP organisms via the Embden-Meyerhof pathway. The acetate then is released and becomes available as substrate to the polyP organisms, and the discussion above for acetate applies.
- (2) PolyP organisms can utilize glucose: In this situation Marais *et al.* could propose biochemical pathways for PHB synthesis from glucose under anaerobic conditions. To do this they assumed that the polyP organisms possess an Embden-Meyerhof pathway.

Thus, either the polyP organisms possess an Embden-Meyerhof pathway (in which event they can take up glucose and store it as PHB in the anaerobic zone) or other

pathways, not yet identified, are operative which can convert acetate to PHB.

This model of Marais *et al.* contributed to the understanding of excess P removal by:

- (1) Discarding the "survival" hypothesis and replacing it by an "advantage" one, i.e. that there is an advantage conferred on the polyP organisms in the anaerobic/aerobic sequencing.
- (2) Introducing bioenergetics to assist in checking the feasibility of any proposed biochemical pathway.
- (3) Proposing that only substrate originating from the influent readily biodegradable COD could be sequestered by the polyP organisms in the anaerobic reactor. In proposing biochemical pathways for the sequestration they encountered difficulties - they were unable to determine whether polyP organisms possess the Embden-Meyerhof pathway or not, and found that, if acetate was the substrate, then established biochemical pathways could not explain PHB formation in the anaerobic zone.

4.4 Further biochemical information

In reviewing the biochemical models presented up to 1983, it is evident that no single model provided an acceptably complete description of the biological excess P removal phenomenon. This situation persisted until 1985. In the interim considerable research was reported corroborating some of the hypotheses built into the models, and generally adding to the knowledge of the excess P removal phenomenon:

Lötter (1983) verified a key proposal of the Rensink (1981) and Marais *et al.* (1983) models by demonstrating that the P released under anaerobic conditions was derived from polyphosphate.

Barnard (1984) observed on full-scale 5-stage modified Bardenpho systems that P was released in the secondary anoxic zone, i.e. under conditions where no substrate was available for storage. He termed this release a 'secondary release'.

Watanabe *et al.* (1984) used scanning electron microscopy combined with energy dispersive X-rays (SEM-EDX) to study the nature of the polyP accumulating organisms. They found cell clusters similar to those identified by Buchan (1981a), but showed that Mg and K, not Ca as found by Buchan, were the

dominant cations in the polyP granules. They proposed that these cations play an important rôle in neutralizing the charges on the polyP. (van Groenestijn and Deinema, 1985, subsequently showed P, K and Mg accumulation in *Acinetobacter* strain 210A).

Miya *et al.* (1984) developed a simplified Schmidt-Thannhauser-Schneider (STS) extraction procedure to determine the various P fractions in sludge. Using this procedure they identified three main P removal mechanisms; normal assimilation, polyphosphate accumulation and mineral phosphate insolubilization. They found that the amount of P removed by normal assimilation was proportional to the volatile solids production and not influenced by anaerobic/aerobic sequencing; P removal by polyphosphate accumulation was increased by anaerobic/aerobic sequencing; P removal by mineral phosphate insolubilization depended on influent characteristics.

Mino *et al.* (1984), using the STS method, fractionated the intracellular P. They identified two types of polyP, low molecular weight and high molecular weight. They showed that low molecular weight polyP principally was responsible for the high P content of biological excess P removal system sludge and was implicated in P release and P uptake. They concluded that low molecular weight polyP served as an energy source under anaerobic conditions, and high molecular polyP as a P source for growth.

Hascoet *et al.* (1984) used Nuclear Magnetic Resonance (NMR) techniques to identify the various forms of P found within the cell. Using NMR, they directly demonstrated the accumulation of polyP under aerobic conditions and its depletion under anaerobic conditions. Further, they demonstrated P release under anoxic conditions on addition of substrate.

Lötter (1984) found that, of one hundred *Acinetobacter* spp. isolated from biological excess P removal systems, fifty-two were capable of nitrate reduction. This indicated that perhaps the organisms mediating biological excess P removal also were implicated in denitrification in nutrient removal systems. (The denitrification capability of *Acinetobacter* subsequently was verified by van Groenestijn and Deinema, 1985). Lötter used the Analytical Profile Index (API) bacterial identification procedure to study the population structure of a 5-stage modified Bardenpho system: When the system was removing P in excess *Acinetobacter* dominated in the mixed liquor, 61 to 66 percent of the organisms

that grew in aerobic cultures were identified to be *Acinetobacter calcoaceticus* var. *lwoffii*; when the P removal was very poor there was only a 10 percent decline in the relative abundance of this organism. Cloete *et al.* (1984), using immunofluorescent techniques, also found "that *Acinetobacter* numbers did not vary regardless of whether phosphate was removed or not." These investigations raised the question as to whether *Acinetobacter* was a genus to be found regularly in mixed liquor of all activated sludge systems, not specific to excess P removal systems. This question was addressed only later.

4.5 P release model

In the absence of a reliable biochemical model Wentzel *et al.* (1984) inaugurated an extensive investigation into the anaerobic phase by studying P release in regard to the readily biodegradable COD. In anaerobic batch tests they found the P release behaviour differed with acetate and with unsettled municipal wastewater as substrate: With acetate a rapid linear increase in P concentration was observed, with wastewater P concentration increased but at a rate that declined continually. This difference in behaviour they proposed to be due to an intermediate step in the sequestration process with wastewater as influent. Accordingly, they accepted the biochemical conversion pathway as proposed by Marais *et al.* and suggested by Fuhs and Chen and Rensink, i.e. conversion of the substrate to acetate (or similar short chain fatty acids) by non-polyP heterotrophs possessing the Embden-Meyerhof pathway in this way to make acetate available to the polyP organisms for sequestration. They also accepted the hypothesis of Marais *et al.* that only the readily biodegradable COD fraction of the wastewater can be converted to short chain fatty acids. However, in the batch tests with wastewater as substrate they could not detect any short chain fatty acids. This, they concluded, was because the rate of conversion by the non-polyP organisms was much slower than the rate of sequestration by the polyP organisms so that any short chain fatty acids would be sequestered immediately, leaving no free acids in solution. Also, the rate of conversion controlled the rate of sequestration and the associated P release. This indicated to them that the kinetics of P release, with sewage as substrate, could be modelled in terms of the rate of conversion of readily biodegradable COD by the non-polyP heterotrophic mass. Their experimental data allowed them to model the conversion *cum* P release as a first order reaction with respect to the active heterotrophic organism mass and the concentration of readily biodegradable COD.

The P release model implied that as the anaerobic mass is increased so the mass of readily biodegradable COD converted will do likewise, but at a declining rate.

Furthermore, being a first order type reaction, the anaerobic mass necessary to convert any selected fraction of the readily biodegradable COD could be reduced by having plug flow or in-series-reactors instead of a single anaerobic reactor. Using the UCT system, they tested the P release model over a wide range of anaerobic mass fractions, in single, series and plug flow anaerobic reactors at different system sludge ages and obtained very good correlation between predicted and observed results.

With regard to the subsequent P uptake, their data showed the magnitude of the P uptake was strongly linked to the magnitude of P release (or equivalently, COD converted) in the anaerobic reactor.

This P release model was applied to the data obtained in the investigation by Siebritz *et al.* (1983) in which single reactors were used with fixed anaerobic mass fractions. This analysis showed that the formulation of Siebritz *et al.* (that P removal was proportional to $[S_{bs}-25]$) was approximately valid for the anaerobic mass fractions used in the Siebritz *et al.* study.

The P release model of Wentzel *et al.*, appeared to provide a general solution for determining the P release for any selected anaerobic mass fraction, sludge age, influent readily biodegradable and total COD concentrations. The P release with wastewater as substrate could be modelled without implicating the polyP organisms *per se*, because the rate of P release was controlled by the conversion action of the non-polyP heterotrophs (with the proviso that the wastewater contains no short chain fatty acids). However, the expected P uptake and P removal still was required to be estimated from empirical relationships between the P release and P uptake. Wentzel *et al.* concluded that this empirical approach was necessary because the polyP organism mass appeared to directly control the rate of P uptake, that is, *modelling of the P uptake would require implicating the polyP organism mass.*

The work of Meganck *et al.* (1984) and Brodisch (1984) supported the conversion hypotheses of Wentzel *et al.* and conclusions; they showed that anaerobic/aerobic systems developed organisms which converted sugars, and similar organic compounds, to short chain fatty acids in the anaerobic stage.

From the work of Wentzel *et al.*, Meganck *et al.* and Brodisch it became apparent that the anaerobic reactor has two primary functions;

- (1) it acts as a fermentation reactor in which the non-polyP facultative

heterotrophs convert a fraction of the influent sewage, readily biodegradable COD, to short chain fatty acids and,

- (2) it provides an environment where the polyP organisms can take up the short chain fatty acids and store these as PHB with associated P release (sequestration).

4.6 Comeau, Hall, Hancock and Oldham model

In 1985 Comeau *et al.* proposed a conceptual model that resolved some of the difficulties that troubled Marais *et al.* (1983). Following Rensink (1981), Comeau *et al.* (1985) accepted that acetate was the substrate and that polyP serves as an energy source for activation of acetate to acetyl-CoA in the anaerobic state. They did not, however, accept the proposals of Marais *et al.* (1983) and Wentzel *et al.* (1984) that the acetate is generated by the conversion of readily biodegradable COD in the anaerobic reactor of the activated sludge system but were of the opinion that the acetate enters the anaerobic reactor with the influent. To overcome the problem encountered by Marais *et al.*, namely that in the anaerobic state with acetate as substrate no source of electrons and protons was available to reduce acetoacetyl-CoA to PHB, they made the novel proposal that these can be supplied from the tricarboxylic acid (Krebs) cycle, i.e. that this cycle could operate also under anaerobic conditions. However, they did not elaborate on how this could be achieved.

It would appear that Matsuo (1985) came to the same solution as Comeau *et al.*; apparently independently he also proposed that the tricarboxylic acid cycle could operate under anaerobic conditions to supply electrons and protons to reduce acetate to PHB (Mino *et al.*, 1987). Unfortunately the publication of his proposals is in Japanese and no translation is available.

Comeau *et al.* noted further that acetate would be taken up by the organism in the undissociated acetic acid (HAc) form, as generally accepted in biochemistry. This causes the proton motive force (pmf) across the cytoplasmic membrane, essential to cell function (for function see Comeau *et al.*, 1985), to be dissipated. To re-establish the pmf they proposed that the polyP serves as the energy source for translocation of protons. They showed further that the cations Mg^{2+} , K^{+} and Ca^{2+} are released with phosphate in the molar ratio cation/phosphate released of 0,27; 0,28 and 0,02 respectively. In terms of charge, one positive cation charge is released with every phosphate.

They described the biochemical pathways only in outline.

In concept, the model of Comeau *et al.* provides a framework for explaining anaerobic/aerobic behaviour of enhanced P release and uptake. However, the model does not provide quantitative information on the pathways, the stoichiometry and the control mechanisms governing the biochemical reactions in this behaviour. Furthermore, the model provides no guidance on the behaviour to be expected under other sets of imposed conditions of substrate and oxygen (or nitrate) tension. Additional information on such other behaviours became available subsequent to the publication of the Comeau *et al.* model.

4.7 Additional information

Two behavioural patterns with regard to the growth of polyP organisms emerged from the research which are of particular significance:

- (1) *Acinetobacter* appear to proliferate not only in anaerobic/aerobic systems removing P in excess but also in completely aerobic systems with no excess P removal.
- (2) In aerobic plate tests *Acinetobacter* can sequester acetate (and store it as PHB) and synthesize polyP at the same time.

In this regard the following information was obtained:

Cloete *et al.* (1984) and Lötter (1984) had observed *Acinetobacter* in systems not exhibiting excess P removal. Lötter and Murphy (1985), in studying population structures of a 5-stage modified Bardenpho system, again demonstrated the dominance of *Acinetobacter* in biological excess P removal systems; however, they also found *Acinetobacter* to be dominant in situations of poor excess P removal by the system and concluded that, although *Acinetobacter* are principally responsible for excess P removal, their mere presence does not indicate that excess P removal is being achieved. Lötter *et al.* (1986) found that even in completely aerobic systems *Acinetobacter* spp. could be abundantly present. This raised the question: Does the *Acinetobacter* species (or strains) in these completely aerobic systems differ from those in systems that exhibited excess P removal? To find an answer to this, Lötter *et al.* (1986) isolated *Acinetobacter* from the completely aerobic systems and showed that

approximately half of these isolates could;

- (1) accumulate polyP with both acetate and glucose as substrate under aerobic culture conditions,
- (2) accumulate PHB in aerobic culture tests with β -hydroxybutyrate as substrate,
- (3) utilize both glucose and acetate as substrate when cultured in an aerobic environment and,
- (4) utilize nitrate as an external electron acceptor, but of these isolates the majority could reduce nitrate to nitrite only.

These results were compared with results obtained from similar tests on *Acinetobacter* isolated from a system exhibiting excess P removal - no significant difference was apparent. These results led Lötter *et al.* to conclude that imposing conditions conducive to excess P removal (by anaerobic/aerobic sequencing) does not stimulate new *Acinetobacter* strains, but rather stimulates polyP and PHB accumulating propensities inherent in strains already present.

In a thorough literature search on the characteristics of *Acinetobacter*, Wentzel *et al.* (1986) found that there was considerable information available in the microbiological and biochemical fields on this organism which apparently had been overlooked by investigators in biological excess P removal. This information can be summarized as follows:

Acinetobacter spp. are ubiquitous in nature and can be readily isolated from soil, water and sewage (Warskow and Juni, 1972; Juni, 1978). All *Acinetobacter* spp. are gram negative, non-motile, catalase positive and oxidase negative (Henriksen, 1973; Juni, 1978; Fricke *et al.*, 1982). The genus requires oxygen for catabolic metabolism, Juni (1978); there are however some species within the genus which can utilize nitrate as an electron acceptor where oxygen is not present (Lötter, 1984). *Acinetobacter* spp. do not possess the Embden-Meyerhof (glycolytic) pathway (Juni, 1978). Instead some species (or strains) possess the Entner-Doudoroff pathway and can metabolize glucose, or similar sugars via this pathway. However, this pathway is not operative under anaerobic conditions.

As a consequence the organisms cannot produce energy from anaerobic fermentation.

4.8 Wentzel, Lötter, Loewenthal, Marais model

Wentzel *et al.* (1986) critically evaluated the previous models that had been proposed, taking due account of the additional information that had become available since the time the Comeau *et al.* model had been proposed. This evaluation can be summarized as follows:

- That *Acinetobacter* do not possess the Embden-Meyerhof pathway implied that the Nicholls *et al.* and Marais *et al.* proposals for PHB synthesis from glucose by the polyP organisms under anaerobic conditions, no longer were feasible.

That some *Acinetobacter* possess the Entner-Doudoroff pathway implied that these organisms can metabolize sugars under aerobic conditions, in contrast to the findings of Fuhs and Chen (1975). This also would explain the presence of *Acinetobacter* in completely aerobic systems, as reported by Lötter *et al.* (1986). Furthermore, Rensink's proposal, that storage of PHB in the anaerobic zone was essential to remove the organisms from competition for substrate in the aerobic zone because their aerobic growth rate was slow, also no longer appeared to be valid.

To overcome these problems, and the problems identified earlier in the Comeau *et al.* model, Wentzel *et al.* set about developing a model that would explain the behaviour of *Acinetobacter* over the total range of substrate and environmental conditions. They accepted the Matsuo/Comeau *et al.* (1985) proposal invoking the tricarboxylic acid (Krebs) cycle in the anaerobic phase behaviour and the importance of maintaining the pmf, but disagreed with the mechanisms proposed by Comeau *et al.* for maintaining the pmf as these led to charge and proton imbalances across the cytoplasmic membrane.

Wentzel *et al.*, after considering the carbon and phosphorus biochemical pathways specific to *Acinetobacter* together with ones accepted as operating generally in microorganisms, identified two key parameters that appeared to regulate these pathways, i.e. the ATP/ADP and NADH/NAD ratios. They proposed detailed biochemical pathways, including the tricarboxylic acid (Krebs) cycle, for the polyP organisms in the anaerobic and aerobic reactors of biological excess P removal systems and described how these pathways are regulated by the ATP/ADP and

NADH/NAD ratios. The proposed pathways met the requirement of maintaining the pmf under anaerobic conditions, and explained how the tricarboxylic acid (Krebs) cycle can operate under anaerobic conditions. Having identified the regulatory mechanisms for the proposed biochemical pathways, i.e. the ATP/ADP and NADH/NAD ratios, Wentzel *et al.* could extend the biochemical model to situations other than the anaerobic/aerobic systems. They found that these ratios are affected by the substrate concentration, intracellular or extracellular (PHB and, say, acetate respectively), and the presence or absence of external electron acceptors (aerobic, anoxic or anaerobic). In terms of the regulatory parameters (ATP/ADP and NADH/NAD) Wentzel *et al.* proposed detailed explanations of the behaviour of polyP organisms over a wide range of conditions. The behaviour proposed can be briefly summarized as follows:

(1) Under aerobic conditions:

- (i) If acetate concentration is high both PHB and polyP storage takes place; this is observed in pure culture tests.
- (ii) If acetate concentration is relatively low *Acinetobacter* spp. grows without PHB or polyP accumulation; this situation is relevant to completely aerobic activated sludge systems.
- (iii) If acetate concentration is very low or zero, but stored PHB is present, polyP accumulation will take place; this situation is relevant to the main aeration basin of anaerobic/anoxic/aerobic systems (modified Bardenpho or UCT).
- (iv) If neither acetate nor PHB is present, maintenance energy is obtained by utilization of substrate generated by the death of *Acinetobacter* spp. (endogenous mass loss); polyP is released uncleaved to the bulk solution proportionally to the protoplasm mass of organisms that died; this situation might be observed explicitly in the reaeration zone (and in the secondary anoxic zone if nitrate is present) and is one component of the so-called secondary release. The P release is not associated with P uptake subsequently.

(2) Under anaerobic conditions:

- (i) If acetate is present, PHB is stored by cleaving stored polyP. Also, maintenance energy is obtained by cleavage of stored polyP.
- (ii) If acetate is not present, irrespective of whether PHB is present or not, maintenance energy is obtained by cleavage of stored polyP. This is another component of secondary release and again is not associated with subsequent P uptake.

(3) Under anoxic conditions (nitrate present):

- (i) If PHB is present but acetate is not, as in the primary anoxic zone, two situations can apply: *Acinetobacter* not able to use nitrate react as in 2(ii); *Acinetobacter* that can use nitrate react as in 1(iii). Hence a release and uptake of P takes place simultaneously giving rise to a net P release or uptake, depending on the relative concentrations of these organisms and their rates of reaction. The energy produced with nitrate is only 2/3 compared with that of oxygen, so that less energy is available under anoxic conditions for polyP synthesis and less P uptake takes place.
- (ii) If neither PHB nor acetate is present, as in the secondary anoxic zone, then: *Acinetobacter* not able to use nitrate react as in 2(ii); *Acinetobacter* that can use nitrate react as in 1(iv). However in the secondary anoxic zone if no nitrate is present, maintenance energy cannot be obtained by oxidation of the protoplasm released due to death; instead it is obtained by cleavage of polyP giving rise to secondary release the same as in 2(ii). When no nitrate is present the protoplasm released due to death may be converted to acetate or other short chain fatty acids by non-polyP facultative organisms, so that P release due to PHB formation can take place and subsequently on aeration give rise to an uptake of P, usually limited. This behaviour has been observed in reaeration zones on occasion.

The model of Wentzel *et al.* appears to provide a comprehensive and consistent explanation of the biological excess P removal phenomenon - all the significant observations reported over the past 30 years appear to find explanation in terms of

this model.

Experimental investigations have verified two key proposals in the model, that ATP is generated from polyP breakdown and that PHB and polyP synthesis/degradation is regulated by the ATP/ADP and NADH/NAD ratios:

Van Groenestijn and Deinema (1987) showed, by using enzymatic studies, that ATP can be generated from polyP breakdown in *Acinetobacter* strain 210A. Lötter and Dubery (1987) also used enzymatic studies to show that key enzymes in the PHB and polyP synthesis and breakdown pathways are regulated by metabolic feedback control, in particular the NADH/NAD and ATP/ADP ratios.

The model also incorporates, and elaborates on, the hypothesis of Comeau *et al.* (1985) and Matsuo (1985) that the tricarboxylic acid (Krebs) cycle operates under anaerobic conditions.

This novel proposal subsequently was investigated experimentally. In an enzyme study on isocitrate dehydrogenase, one of the cycle enzymes, Lötter (1988) showed that this enzyme still was operative under anaerobic conditions albeit at a reduced level. The proposed operation of the tricarboxylic acid cycle under anaerobic conditions also explains observations made previously by Florentz and Hartemann (1982). They showed that two tricarboxylic acid cycle enzymes, fumarase and citrate synthase, remained operative under anaerobic conditions. These investigations provide substantive support for the Comeau/Wentzel *et al.* biochemical model.

4.9 Mino, Arun, Tsuzuki, Matsuo model

The biochemical models of Matsuo, Comeau *et al.* and Wentzel *et al.* presume that the organisms do not possess the Embden-Meyerhof pathway. Mino *et al.* (1987) proposed a biochemical model for polyP organisms that possess an Embden-Meyerhof pathway receiving acetate as substrate under anaerobic conditions. From an experimental enquiry they found that under *anaerobic* conditions acetate was taken up and stored as PHB, that P was released and that intracellular carbohydrate was utilized. Under *aerobic* conditions, they found that PHB was utilized, that P was taken up and that intracellular carbohydrate was formed. To explain the observed behaviour, they proposed a conceptual model that is in agreement with the Comeau/Wentzel *et al.* model except in the source of NADH₂ for PHB synthesis under anaerobic conditions: With the Embden-Meyerhof pathway operative the

intracellular carbohydrate is consumed under anaerobic conditions via the Embden-Meyerhof pathway to produce NADH_2 for reducing acetate to PHB, there is no need to implicate the tricarboxylic acid (Krebs) cycle. The intracellular carbohydrate then is reformed under the subsequent aerobic conditions via a 'reversal' of the Embden-Meyerhof pathway.

The model of Mino *et al.* resolves the difficulty Marais *et al.* encountered in describing a feasible pathway for PHB synthesis with acetate as substrate under anaerobic conditions. However, the Mino *et al.* model very likely does not apply for *Acinetobacter* spp. because these organisms do not possess the Embden-Meyerhof pathway (Juni, 1978; Bergey, 1984).

4.10 Kinetics of excess P removal

With the development of the models above it would appear that, in the main, the biochemistry of the biological excess P removal phenomenon had been resolved. This caused that the research attention shifted to investigating the kinetics of excess P removal. The biochemical models are, by themselves, not sufficient to develop a kinetic model for the P release and uptake as they supply no information on kinetic behaviour.

The kinetics of P release under anaerobic conditions with sewage as substrate was described earlier (Wentzel *et al.*, 1984). They showed that the kinetics of the P release could be formulated without recourse to the polyP organism *per se*. With regard to P uptake in the subsequent aerobic zone, they found that kinetic formulation required direct incorporation of the polyP organisms, with their growth and death characteristics. However in the mixed cultures, normal to activated sludge systems, Wentzel *et al.* could not isolate these characteristics for reason that the response of the other heterotrophic organisms either obscured or swamped out that of the polyP organisms. Consequently, they could only develop empirical relationships for the uptake. For kinetic formulation, Wentzel *et al.* (1987) concluded, it was essential that systems be developed in which polyP organisms dominate.

Wentzel *et al.* (1988a) found that *pure* cultures could not be used to gain information on the kinetics of the excess P removal processes and the compounds associated with these processes: Analysis of the data in the literature on pure culture studies of *Acinetobacter* spp. (e.g. Abbott *et al.* 1973, Du Preez 1980) indicated that the responses of these organisms in pure cultures were very different from the responses

inferred from observations in activated sludge systems. For example, with acetate as substrate in aerobic pure culture chemostat studies, the maximum specific growth rate constant was extremely high ($\hat{\mu} = 4\text{-}30/\text{d}$, cf. $2\text{-}4/\text{d}$ for activated sludge) so also the specific endogenous mass loss rate ($b = 1,0/\text{d}$, cf. $0,24/\text{d}$ for activated sludge). Wentzel *et al.* came to the conclusion that instead of pure cultures, *enhanced* cultures should be developed as these cultures may be more relevant and supply the required information. By enhanced culture they meant: Development of a polyP organism culture by selecting a substrate and set of environmental conditions that favour polyP organisms to the extent that these become the dominant primary organisms and dominate the culture response. Growth of competing normal heterotrophic organisms will be curtailed naturally but not deliberately terminated, neither will predation by higher organisms and other interactive effects be positively excluded. Also, a strain (or strains) of polyP organism will be naturally selected that may differ from that artificially selected and grown in pure cultures.

From the biochemical model Wentzel *et al.* identified sets of conditions that give rise to excess P removal processes. One set is to subject the organism mass to an anaerobic/aerobic sequence with short chain fatty acids fed to the anaerobic phase, conditions present in the Phoredox, modified Bardenpho and UCT systems with acetate as influent. Accordingly, in UCT and modified Bardenpho systems, by starting with 100 percent municipal wastewater as influent, incrementally decreasing the wastewater fraction and increasing the acetate fraction, Wentzel *et al.* developed enhanced cultures of polyP organisms. In these systems, the API identification procedure indicated that greater than 90 percent of the organisms were *Acinetobacter* spp.

The enhanced cultures gave growth and endogenous mass loss characteristics for the enhanced polyP organism mass that appear to be close to those inferred from the activated sludge studies using sewage as influent on UCT systems. For example, specific endogenous mass loss rate $b = 0,04/\text{d}$, as inferred by Wentzel *et al.* (1984).

From experimental studies using the enhanced cultures, Wentzel *et al.* (1988b);

- (1) identified the compounds influenced by biological excess P removal,
- (2) identified the processes that act on these compounds, and

- (3) conceptualized a mechanistic model that qualitatively describes the kinetic and stoichiometric behaviour of the processes and compounds.

They identified 12 compounds, associated with 13 processes, as being directly involved with biological excess P removal. They grouped the processes that act on the compounds into three broad categories; sequestration of acetate, growth and endogenous mass loss. They developed a detailed conceptual mechanistic model of the processes, and their stoichiometric interaction with the compounds.

Using the mechanistic model as a basis, Wentzel *et al.* (1988c) formulated the process rates mathematically, so also the stoichiometric relationships between the processes and compounds, and incorporated these into a kinetic model of biological excess P removal. To calibrate the model, Wentzel *et al.* determined the stoichiometric and kinetic constants from the enhanced culture systems and batch tests on mixed liquors from these systems. They compared the predicted behaviour of the various compounds with that observed in the different batch tests and concluded that the closeness with which the predictions conform to the observations over the wide range of conditions in the tests constitutes evidence for the acceptability of the model.

From the model and experimental observations Wentzel *et al.* concluded that the polyP and the non-polyP populations act relatively independently of each other:

For some reason, as yet not understood, in mixed cultures the polyP organisms are not predated whereas the non-polyP ones suffer heavy predation. The only sources of interaction appear to be;

- (1) in the anaerobic phase where the short chain fatty acids generated by the non-polyP organisms are sequestered by the polyP organisms and,
- (2) in the endogenous mass loss of the polyP organisms where soluble endogenous COD generated can be utilized by the non-polyP organisms.

This lack of interaction, between polyP and non-polyP populations, most likely makes that fusion of the kinetic models describing the behaviour of the two populations at a future date, will not present undue difficulties. Competition between the populations groups for the same substrate, although it influences the solution, will not influence the structure of the joint model because this is governed

by kinetics not by interaction.

4.10 Discussion

The main achievements of the third period can be summarized as follows:

- (1) Development of techniques to quantify the various P fractions in the sludge.
- (2) Development of a biochemical model that describes the behaviour of the organisms mediating biological excess P removal for a wide variety of substrate and environmental conditions.
- (3) Identification of the conversion of readily biodegradable COD in the anaerobic reactor, and mathematical formulation of the kinetics of this process, and of the P release in terms of this conversion.
- (4) Development of enhanced cultures of polyP organisms in the UCT and modified Bardenpho systems, yielding mixed liquor with the polyP organism *Acinetobacter* spp. in excess of 90 percent.
- (5) Identification of the processes that are involved in the biological excess P removal phenomenon, and the compounds associated with these processes.
- (6) Incorporation of the processes and compounds into a kinetic model describing the behaviour of polyP organisms in enhanced cultures.

5. CONCLUSIONS

This chapter has traced the evolution of biological excess phosphorus removal from the first observation of the phenomenon in activated sludge systems to the present. Understanding of the phenomenon has grown from the rudimentary empirical explanations to highly sophisticated models involving complex biochemical and kinetic descriptions. With the fund of understanding now available, the biological excess P removal phenomenon can be applied with confidence to design systems that will operate optimally insofar as the P removal aspect is concerned. However, operating experience with full-scale systems has brought to light other problems that appear to be specific to these systems:

- (1) At the long sludge ages common to nitrification/denitrification/P removal systems, there is a proneness to produce bulking sludges, due to the

proliferation of filamentous organisms.

- (2) In many wastewaters there is a shortage of readily biodegradable substrate so that the P removal may be inadequate; the supply of short chain fatty acids by acid fermentation of primary sludge is one solution, but the design, operation and performance of these fermentation units is virtually unexplored.
- (3) The liquor of anaerobically digested waste activated sludge from UCT and modified Bardenpho systems contains high concentrations of PO_4^{3-} , Mg^{2+} and NH_4^+ ; these ions can combine and precipitate as struvite, particularly in pipe bends, causing blockages.

Future research will be increasingly directed towards solving these problems.

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CHAPTER 3

KINETICS OF BIOLOGICAL PHOSPHORUS RELEASE

1. INTRODUCTION

From a review of literature on biological excess phosphorus (P) removal Marais *et al.* (1983) summarized the observed behavioural characteristics of this phenomena as follows:

- (1) P release under anaerobic conditions must be observed in order to give rise to P uptake subsequently under aerobic or anoxic conditions, which results in a nett P removal for the system.
- (2) For any fixed process configuration the magnitude of the excess P removal is linked directly to the magnitude of the readily biodegradable (readily assimilable) influent COD concentration, S_{bsi} ; the higher the S_{bsi} , the higher the P release, uptake and removal. In contrast, the slowly biodegradable influent COD, S_{bpi} , appears to have little direct influence on excess P removal.
- (3) The greater the fraction of total sludge mass in the anaerobic reactor, f_{xa} , the greater the nett P removal.
- (4) Nitrate recycled to the anaerobic reactor has an adverse effect on P removal; the greater the mass of nitrate recycled, the lower the P removal.

Marais *et al.* (1983), taking note of the observation that the P removal is strongly linked to the readily biodegradable COD fraction of the influent, considered the polyP organism behavioural pattern for two types of readily biodegradable COD, namely (1) short chain fatty acids or (2) 'glucose like' materials. They put forward the following hypothesis for polyP organism behaviour with each of these readily biodegradable COD substrate types:

- (1) Short chain fatty acids: In an anaerobic/aerobic system, if polyP organisms are discharged to an anaerobic reactor – a condition devoid of nitrate or oxygen – in which short chain fatty acids are present, the polyP organisms break down (hydrolyze) stored polyphosphate chains, releasing phosphate to the surrounding liquid. The bond energy thus released is utilized (by a

pathway not yet understood) to absorb, complex and store short chain fatty acids within the organism, in this fashion partitioning or sequestering substrate for its exclusive use. The polyP organisms with the sequestered substrate, on leaving the anaerobic zone and entering one where there is an external electron acceptor, can grow in such a reactor without being in competition with the non-polyP organisms. The polyP organisms utilize the sequestered substrate for two purposes, for (i) replenishment of the polyP pool by abstracting P from the surrounding medium, and (ii) growth. Thus under anaerobic/aerobic sequencing, by having the propensity to store polyphosphate, the polyP organisms gain a positive advantage over the non-polyP organisms and can proliferate under such conditions.

- (2) 'Glucose-like' materials: When glucose is the readily biodegradable COD, Marais *et al.* considered two situations; either the polyP organisms can utilize the glucose anaerobically, or, they cannot. If the polyP organisms can utilize glucose, they proposed that, in the anaerobic reactor, the polyP organisms take up the glucose, pass it through the Embden-Meyerhof pathway to form acetate, which then is stored in the same fashion as the short chain fatty acids, as discussed above. If the polyP organisms cannot utilize glucose anaerobically (i.e. do not possess the Embden-Meyerhof pathway), Marais *et al.* proposed that in the anaerobic zone some of the non-polyP heterotrophs (facultative aerobes), absorb the readily biodegradable COD and derive some energy via the glycolytic (Embden-Meyerhof) pathway by transforming substrates like sugars to short chain fatty acids and similar forms which are released to the surrounding medium. These materials then are available to the polyP organisms for sequestration, as described in (1) above; thus, when the wastewater does not contain lower fatty acids, the non-polyP heterotrophs may be indirectly implicated in excess P removal.

Marais *et al.* also noted that readily biodegradable COD, S_{bs} , appears to consist of molecules that can pass directly through the cytoplasmic membrane of the heterotrophs; molecules that cannot do so, the slowly biodegradable particulate COD, S_{bp} , require adsorption and extracellular breakdown to smaller units before entering the organism. The breakdown of S_{bp} is relatively slow giving rise to a utilization rate approximately one tenth of that for S_{bs} utilization (Marais and Ekama, 1976); in consequence S_{bp} should have little effect on P release.

Using these ideas Siebritz *et al.* (1983) devised an empirical model that, in the main, reflects the P removal behavioural pattern observed. In their model, the effects of the anaerobic mass fraction, f_{xa} , and available readily biodegradable COD¹, S_{bsa} , are formulated as a multiplicative function with an appropriate constant chosen such that the removal is predicted approximately correctly. No cognizance is taken of P release and uptake *per se*; only the nett effect – P removal – is modelled.

Clearly the Siebritz *et al.* model is restrictive and empirical. To model P removal it would appear essential to include P release and P uptake. This chapter will deal principally with P release, its kinetics and the factors affecting the kinetics.

2. PRELIMINARY INVESTIGATIONS

2.1 Short chain fatty acid content of sewage

In developing a hypothesis for polyP organism behaviour Marais *et al.* proposed that the readily biodegradable COD could consist of two substrate types, short chain fatty acids and glucose-like materials. Accordingly it was decided to measure the readily biodegradable COD and short chain fatty acids in the sewage being used as influent to the experimental units. Batches of unsettled sewage were obtained from the Mitchell's Plain sewage treatment works (Cape Town) and stored in stainless steel tanks at 4°C. Daily, for a period of 1-2 weeks, a sample of sewage was drawn and the total COD, readily biodegradable COD and short chain fatty acids concentrations were determined. Short chain fatty acids measured were acetic, propionic, isobutyric, n-butyric, 2-methylbutyric, 3-methylbutyric and n-valeric.

The mean values for total COD (S_{ti}), readily biodegradable COD (S_{bsi}), acetate concentrations and the $COD(acetate)/S_{ti}$ and $COD(acetate)/S_{bsi}$ ratios for four batches of sewage are shown in Table 3.1, together with values of the standard deviation of the means. Only the concentration of the short chain fatty acid acetate is recorded as none of the other short chain fatty acids were present in significant quantities. It should be noted that during the two weeks of storage of any particular sewage batch at 4°C no significant changes in total COD, readily biodegradable COD or acetate concentration were measured. The means of the $COD(acetate)/S_{bsi}$ and $COD(acetate)/S_{ti}$ ratios for the four batches of sewage, were calculated to be 0,038 mgCOD/mgCOD and 0,21 mgCOD/mgCOD respectively with standard

¹By available is meant the S_{bs} concentration in the anaerobic reactor after the loss due to oxidation with NO_3^- as electron acceptor and the dilution effect of the recycles have been taken into account.

Table 3.1: Total COD, S_{bs} and acetate concentration of raw municipal sewage obtained from Mitchell's Plain Treatment Works, Cape Town.

Sewage Batch	Total COD (mgCOD/l)	S_{bs} (mgCOD/l)	Acetate (mgHAc/l)	$\frac{\text{Acetate}}{S_{bs}}$	$\frac{\text{Acetate}}{S_{ti}}$
	$\bar{X}^* \quad SD_{\bar{X}}^{**}$	$\bar{X} \quad SD_{\bar{X}}$	$\bar{X} \quad SD_{\bar{X}}$	(mgCOD/ mgCOD)	(mgCOD/ mgCOD)
1	985 14,3	203 7,5	29,3 8,5	0,15	0,032
2	988 14,1	181 14,8	39,2 2,3	0,23	0,040
3	779 8,4	114 4,9	22,2 4,7	0,21	0,029
4	734 10,7	129 5,0	36,4 0,4	0,28	0,050

* \bar{X} = the mean of a number of samples from each batch of sewage

** $SD_{\bar{X}}$ = standard deviation of the mean

deviations of the means of 0,0046 and 0,027. Thus, the concentration of short chain fatty acids, in this case only acetate, constituted about 20 percent of the readily biodegradable COD or 4 percent of the total COD. From these experiments it is apparent that the readily biodegradable COD fraction of Mitchell's Plain unsettled sewage consisted mainly of the 'glucose-like' materials.

2.2 P release experiments

To inaugurate enquiry into the P release phenomenon a series of batch tests was performed as follows: Sludge was taken from the aerobic zone of a UCT system and aerated in a batch reactor for 30 minutes, to ensure complete utilization of available substrate; the batch then was left for 3 hours to reduce all oxygen and nitrate present, with the contents being thoroughly flushed with nitrogen gas. Thereafter a selected substrate type was added with nitrogen gas flushing continuing, and the pH being controlled to $\pm 7,4$ with addition of acidity or alkalinity. Temperature was controlled at 20°C in these and all subsequent experiments. Total P concentration on filtered samples was monitored throughout the test. Three sets of tests were undertaken, with (1) acetate, (2) glucose and (3) municipal sewage addition respectively.

- (1) Acetate addition: The observed P release-time profiles are shown in Fig 3.1(a). Evidently (i) the P profiles consisted of two well-defined parts, an initial linear increase in P concentration to some value, after which no significant further increase was observed; (ii) rate of P release was independent of the concentration of acetate added; and (iii) mass of phosphorus released was proportional to the mass of acetate added [Fig 3.1(b)]. Batch tests at different sludge concentrations (not shown) gave higher release slopes at higher sludge concentrations.

From Fig 3.1(b) evidently the energy requirements in terms of P release for sequestration of the acetate was in the ratio $\Delta P : \Delta \text{COD}(\text{acetate}) = 5:20 = 1:4$, i.e., the energy from release of one mgP was required to sequester 4 mgCOD(acetate). However from a number of such tests $\Delta P : \Delta \text{COD}(\text{acetate})$ fluctuated between about 1:2 and 1:5 mgP/mgCOD, so that no firm expected value could be established.

- (2) Glucose addition: Response to glucose addition is shown in Fig 3.2; evidently glucose did not induce P release. However, when the influent to the system from which the sludge sample was taken was augmented by

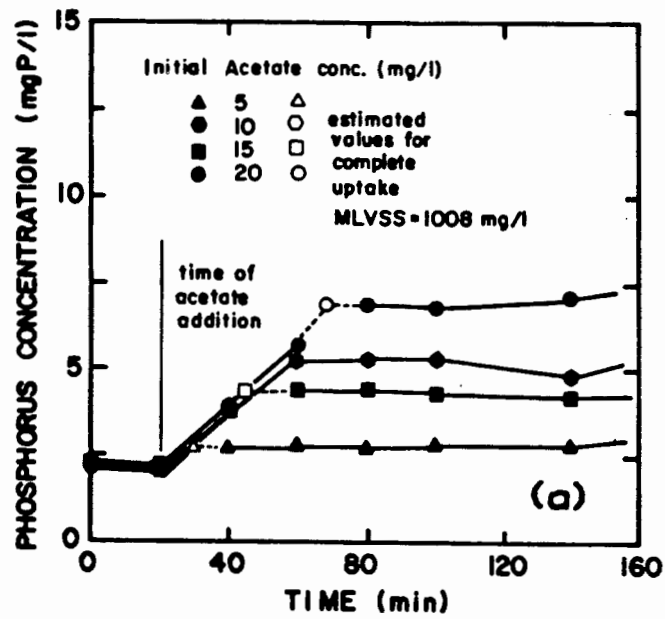


Fig 3.1(a): Phosphorus concentration–time profiles for phosphorus release in a batch anaerobic reactor with different concentration of acetate added.

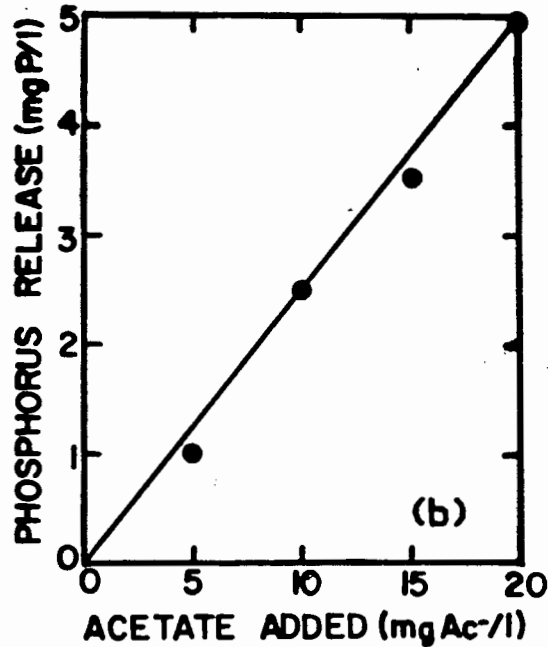


Fig 3.1(b): Magnitude of phosphorus release versus initial concentration of acetate added for data in Fig 3.1(a).

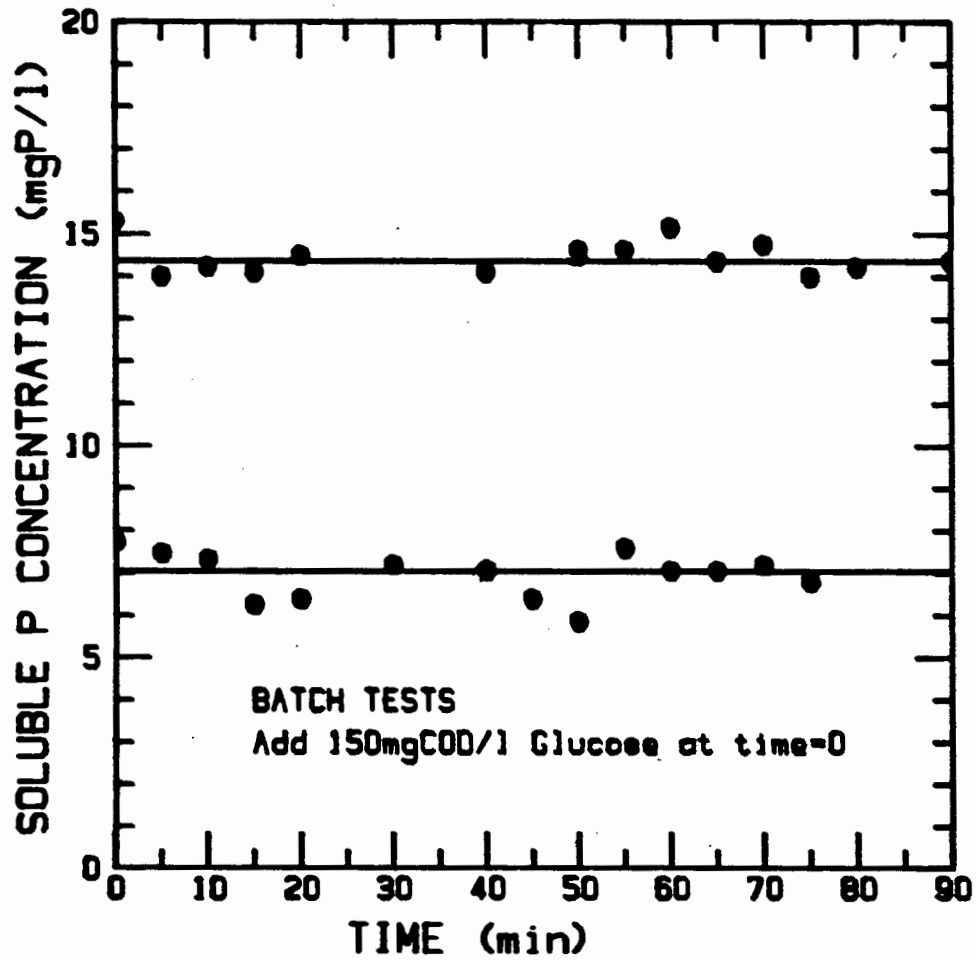


Fig 3.2:

Phosphorus-time profile in an anaerobic batch test with glucose addition at time = 0 [mixed liquor drawn from a plant receiving municipal wastewater, not acclimatized to glucose].

glucose addition, a propensity to utilize glucose in the anaerobic reactor developed in time, and this was reflected in batch tests with glucose addition to mixed liquor samples taken from the system. Unfortunately no details of these tests were recorded.

- (3) **Sewage addition:** Typical examples of the P-time profiles with addition of sewage containing a measured S_{bsi} fraction, are shown in Fig 3.3(a). All the tests characteristically showed a P release at a rate which declined as the test progressed. It was found that the P release-time plot could be modelled very closely as a first order relationship,

$$P_t = P_{\max} [1 - ((P_{\max} - P_o)/P_{\max}) e^{-Ct}] \quad (3.1)$$

where

P_o = the initial P concentration

P_{\max} = the maximum potential P concentration

P_t = P concentration at time t

$(P_{\max} - P_o)/C$ = ΔP_{\max} = the maximum potential release of P possible
= first order rate constant.

ΔP_{\max} and C were obtained by trial curve fitting of Eq (3.1) to the data in Fig 3.3(a). For each test, the fit was checked graphically by plotting $\log(P_{\max} - P_t)$ versus time which should plot to a straight line having a slope of $-C/2.303$ and an intersection of $P = \Delta P_{\max}$, see Fig 3.3(b). The straight line plot confirms the first order relationship. From a number of such experiments an interesting feature became apparent, that the ratio $\Delta P_{\max}:S_{bsi}$ ranged around 1.2mgP/mgCOD, i.e. one mgP needs to be released to supply energy to sequester 2 mgCOD of readily biodegradable substrate.

The experiments described above for sewage were of the batch type. To check the results the anaerobic reactor of the UCT system was converted first to operate in a plug flow mode and thereafter in a four-in-series reactor mode. A typical set of data for the plug flow system is shown in Fig 3.4(a), and for the series system in Fig 3.5(a). Corresponding $\log(P_{\max} - P_t)$ plots are shown in Figs 3.4(b) and 3.5(b) respectively. Again it is apparent that the P release behaviour with sewage conformed to a first order process. From the plug flow data again the P release potential ΔP_{\max} with respect

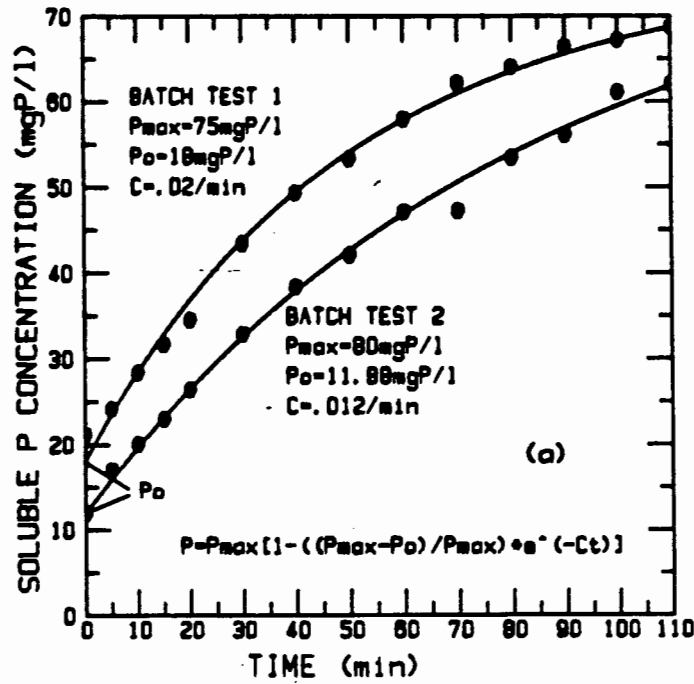


Fig 3.3(a):

Phosphorus concentration with time in an anaerobic batch test on addition of unsettled municipal wastewater. First order fit is demonstrated using Eq (3.1).

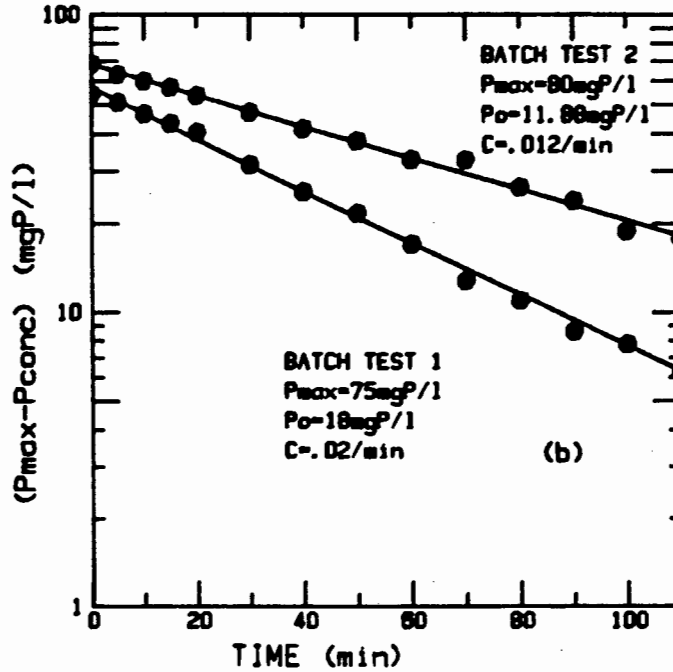


Fig 3.3(b):

Data in Fig 3.3(a) shown plotted $\log(P_{\max}-P_t)$ versus time. First order nature is demonstrated by the straight line of the plot. Line fitted using Eq (3.1) with constants from Fig 3.3(a).

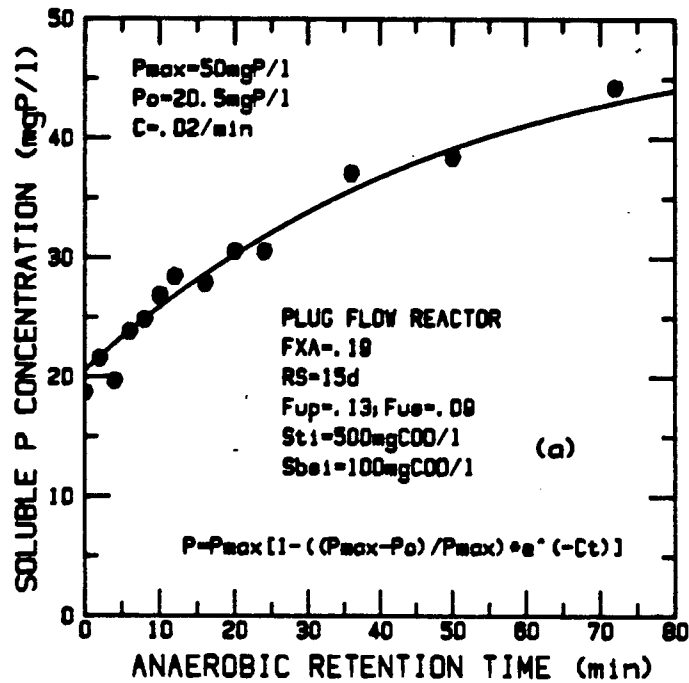


Fig 3.4(a):

Phosphorus release in a plug flow anaerobic reactor of a UCT system with unsettled municipal wastewater as influent. First order fit is demonstrated using Eq (3.1).

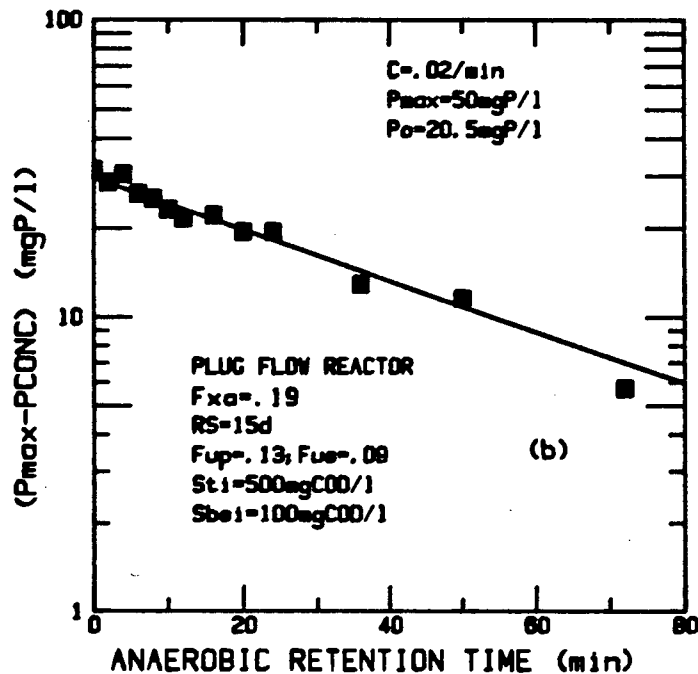


Fig 3.4(b):

Data in Fig 3.4(a) shown plotted $\log(P_{max} - P_t)$ versus time. First order nature is demonstrated by the straight line of the plot. Line fitted using Eq (3.1) with constants from Fig 3.4(a).

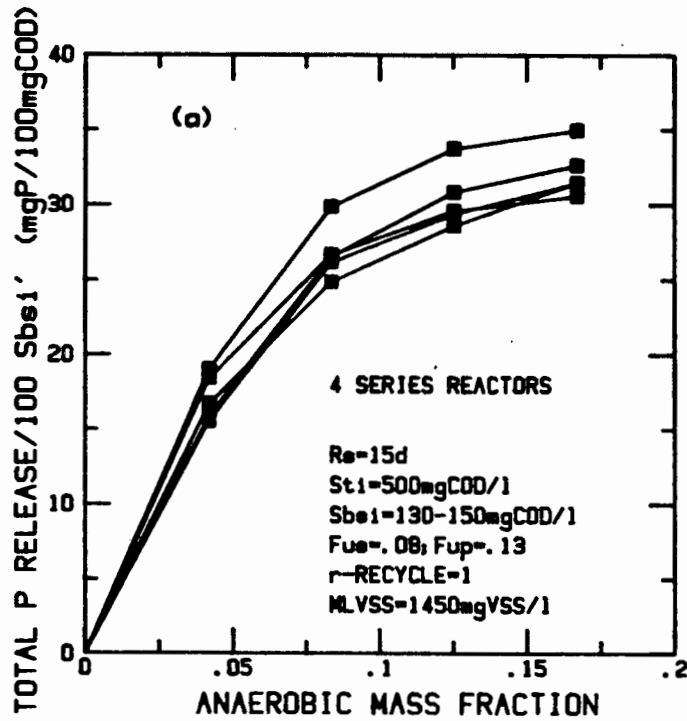


Fig 3.5(a): Cumulative phosphorus release versus cumulative anaerobic mass fraction in a four-in-series anaerobic reactor of a modified UCT system with unsettled municipal wastewater as influent.

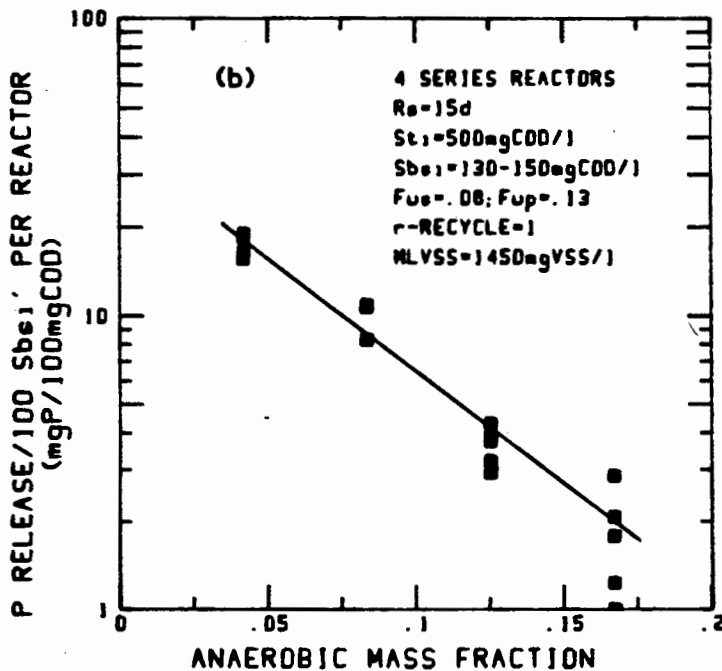


Fig 3.5(b): Data in Fig 3.5(a) shown plotted $\log(P \text{ release per reactor})$ down series versus cumulative anaerobic mass fraction.

to S_{bsi} was in the ratio 1:2 mgP/mgCOD.

From the experimental results the following conclusions appear to be indicated:

- (1) With acetate addition the rate of P release is independent of the acetate concentration, i.e. the reaction is zero order with respect to acetate concentration, and the mass of P released is proportional to the mass of acetate added.
- (2) With glucose addition it would appear that the polyP organisms could not utilize glucose *per se*; either the glucose needed to be modified by the non-polyP organisms to a form suitable for utilization by polyP organisms, or, polyP organism types needed to be selected into the system that can metabolize the glucose. Both alternatives require time to develop.
- (3) With sewage addition in batch tests and steady state systems, the kinetics of P release is of a first order form, not zero order as with acetate addition. Furthermore, the mass of P released appears to be proportional to the mass of readily biodegradable COD added. It would appear that the readily biodegradable COD requires modification in order to become available for sequestration by the polyP organisms, with associated P release.

From the conclusions above it is apparent that readily biodegradable COD requires modification before sequestration can occur. It is hypothesized that this modification is the conversion of the readily biodegradable COD to short chain fatty acids by the non-polyP heterotrophs, as suggested by Marais *et al.* (1983). Comparing the rates of P release with acetate and with sewage, it would seem that the rate of short chain fatty acid sequestration by the polyP organisms, with associated P release, is much higher than the rate of short chain fatty acid generation by the non-polyP organisms. This implies that;

- (1) P release is controlled by the rate of conversion and,
- (2) the P release rate is controlled by the action of the non-polyP organism mass.

These conclusions form the basis for the development of a mathematical model for anaerobic P release *with sewage as substrate*.

3. MODEL DEVELOPMENT

From the behavioural pattern of P release in the anaerobic reactor the following model is proposed². It is hypothesized that:

- (1) Only readily biodegradable COD (S_{bs}) can be converted to a form suitable for sequestration by the polyP organisms ($X_{B,G}$) within the time scale of residence of the mixed liquor in the anaerobic reactor.
- (2) The conversion is mediated by the non-polyP heterotrophic mass in the anaerobic zone, ($X_{B,Hn}$).
- (3) All S_{bs} converted is immediately sequestered by the polyP organisms, i.e. the rate of P release is controlled by the rate of conversion.
- (4) All S_{bs} not converted in the anaerobic reactor is utilized subsequently for non-polyP heterotrophic growth.
- (5) Any nitrate recycled to the anaerobic reactor is utilized by the non-polyP heterotrophic mass, reducing the amount of S_{bs} available for conversion as follows:

$$S'_{bsi} = S_{bsi} - r_{8,6} N_{O3,r} \quad (3.2)$$

where S'_{bsi} = Readily biodegradable COD available for conversion per litre influent (mgCOD/l)

S_{bsi} = Readily biodegradable influent COD concentration (mgCOD/l)

$N_{O3,r}$ = Nitrate concentration in recycle to anaerobic reactor (mgN/l)

r = Recycle ratio based on influent flow

$8,6$ = Mass of COD removed per unit nitrate denitrified in synthesis [mgCOD/mg(NO_3 -N)].

- (6) The rate of conversion of S_{bs} is given by

$$dS_{bs}/dt = -K X_{B,Hn} S_{bs} \quad (3.3)$$

²Details of the symbol system used in the modelling are given in Appendix A.

where K = first order rate constant (d^{-1})
 S_{bs} = readily biodegradable COD concentration, (mgCOD/ ℓ).

- (7) The rate of P release is assumed to be stoichiometrically related to the S_{bs} sequestered. Hence

$$\begin{aligned} dP/dt &= -C_{sp} (d S_{bs}/dt) \\ &= C_{sp} K X_{B,Hn} \end{aligned} \quad (3.4)$$

where C_{sp} = stoichiometric ratio ($\Delta P:\Delta S_{bs}$)
 $= 0.5 \text{ mg}(\text{PO}_4\text{-P})/\text{mgCOD converted.}$

Consider a single anaerobic reactor with volume V , influent flow rate Q and recycle ratio r , as shown in Fig 3.6(a). A mass balance on S_{bs} yields

$$V dS_{bs}/dt = \left(Q S_{bsi}^{in} \right) - \left((1+r) Q S_{bs}^{out} \right) - \left(K X_{B,Hn} S_{bs} V dt \right) \quad (3.5)$$

Hence, at steady state ($dS_{bs}/dt = 0$):

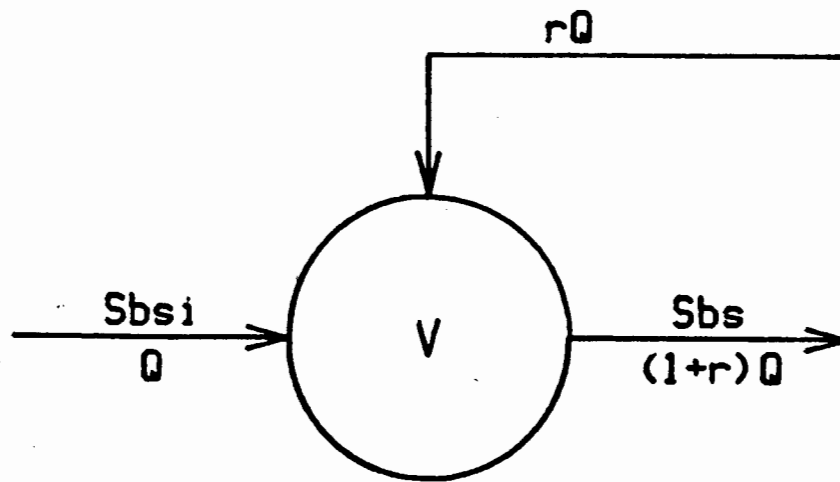
$$S_{bs} = \frac{S_{bsi}' / (1 + r)}{(1 + K X_{B,Hn} R / (1 + r))} \quad (3.6)$$

where R = nominal retention time of anaerobic reactor
 $= V/Q$
 Q = Influent flow to system (ℓ/day)
 V = Volume of reactor (ℓ).

Similarly, from a mass balance on the n^{th} reactor in a series of N anaerobic reactors of equal volume, the S_{bs} concentration is given by

$$S_{bsn} = \frac{S_{bsi}' / (1 + r)}{(1 + K X_{B,Hn} R_N / (1 + r))^n} \quad n = 1, 2, \dots, N \quad (3.7)$$

where $R_1 = R_2 = \dots R_N$

**Fig 3.6(a):**

Schematic diagram of a single anaerobic reactor (with volume, V) receiving influent flow (with flow rate, Q) and r -recycle flow (with recycle ratio, r). The influent readily biodegradable COD (with concentration, S_{bsi}) is converted in the anaerobic reactor to short chain fatty acids by the non-polyP heterotrophs. The readily biodegradable COD not converted (with concentration, S_{bs}) leaves the reactor via the outflow (with flow rate, $(1+r)Q$).

Concentration of non-polyP heterotrophs in the anaerobic reactor, $X_{B,Hn}$, is given by

$$X_{B,Hn} = f_{xa} \cdot MX_{B,H} / V_{at} \quad (3.8)$$

where V_{at} = total anaerobic volume (ℓ)
 f_{xa} = anaerobic mass fraction
 $MX_{B,H}$ = active mass of non-polyP organisms in system (mgVASS)
 V_{aN} = volume of each anaerobic reactor (ℓ)

Using Eq (3.8) and noting that $V_{at} = N \cdot V_{aN}$ and $R_N = V_{aN}/Q$, substituting in Eq (3.7) gives

$$S_{bsn} = \frac{S'_{bsi} / (1 + r)}{(1 + K \frac{f_{xa} MX_{B,H}}{N Q} / (1 + r))^n} \quad (3.9)$$

where $MX_{B,H}$ develops from the total mass of biodegradable influent COD less the mass of COD sequestered by the polyP organisms and $MX_{B,H}/Q$ is the mass developing per litre influent flow,

$$\frac{MX_{B,H}}{Q} = \frac{[S_{bi} - (S'_{bsi} - (1 + r) S_{bsN})] Y_H R_s}{(1 + b_H R_s)} \quad (3.10)$$

where S_{bi} = biodegradable influent COD (mgCOD/ ℓ)
 Y_H = heterotrophic organism yield constant (0,45 mgVASS/mgCOD)
 b_H = heterotrophic endogenous mass loss rate constant
 (0,24 mgVASS/mgVASS/d at 20°C)
 R_s = system sludge age (d)
 S_{bsN} = readily biodegradable COD concentration leaving the last anaerobic reactor (mgCOD/ ℓ)

The P release in the n^{th} reactor (per litre influent flow), ΔP_n , is derived from Eqs (3.4 and 3.9), i.e.

$$\Delta P_n =$$

$$C_{sp} S_{bsi} \left[\frac{1}{\left(1 + K \frac{f_{xa}}{N} \frac{MX_{B,H}}{Q} / (1+r) \right)^{(n-1)}} - \frac{1}{\left(1 + K \frac{f_{xa}}{N} \frac{MX_{B,H}}{Q} / (1+r) \right)^n} \right] \quad (3.11)$$

4. EXPERIMENTAL VERIFICATION

In order to test the theory set out above on biological excess P removal systems a number of modified UCT systems were operated over a period of about one year. Units were run at system sludge ages of 8, 10, 15, 20 and 28 days (maintained by hydraulic control of sludge age) with an unsettled municipal wastewater influent COD as near as possible to 500 mgCOD/l. The TKN/COD ratio ranged from 0,08 to 0,13 mg(TKN-N)/mgCOD. The influent P was augmented, by KH_2PO_4 addition, to 15 or 20 mg(PO_4 -P)/l to ensure that P was always present in the effluent. The anaerobic zone consisted of either a single reactor or two or four reactors in series, with total f_{xa} ranging from 0,09 to 0,356. Recycle ratio from the anoxic to the anaerobic zone was either one or two; underflow recycle was either 0,75 or one; internal recycle from the aerobic to the secondary anoxic zone was either four or two. The general layout of the systems is shown in Fig 3.6(b). The S_{bsi} was measured daily using the method described in the WRC Manual (1984).

The following tests were done daily: (1) unfiltered influent COD, (2) filtered effluent COD, (3) unfiltered influent TKN, (4) filtered effluent TKN, (5) unfiltered influent total phosphate, (6) filtered effluent total phosphate, (7) total phosphate on filtered mixed liquor samples from all the reactors, (8) filtered effluent nitrate, (9) nitrate on filtered mixed liquor samples from all the reactors, (10) oxygen utilization rate of the aerobic reactor, (11) pH of the aerobic reactor, (12) VSS of the aerobic mixed liquor.

Each batch of sewage supplied feed for approximately two weeks, the batches being stored at 4°C. (The sewage remained unaffected by this storage, see section 2.1 above.) Batches often differed quite appreciably with respect to their TKN/COD ratios and S_{bsi} concentrations. Data obtained on each batch of sewage were averaged and these average values used to evaluate the theory. Typical data on the

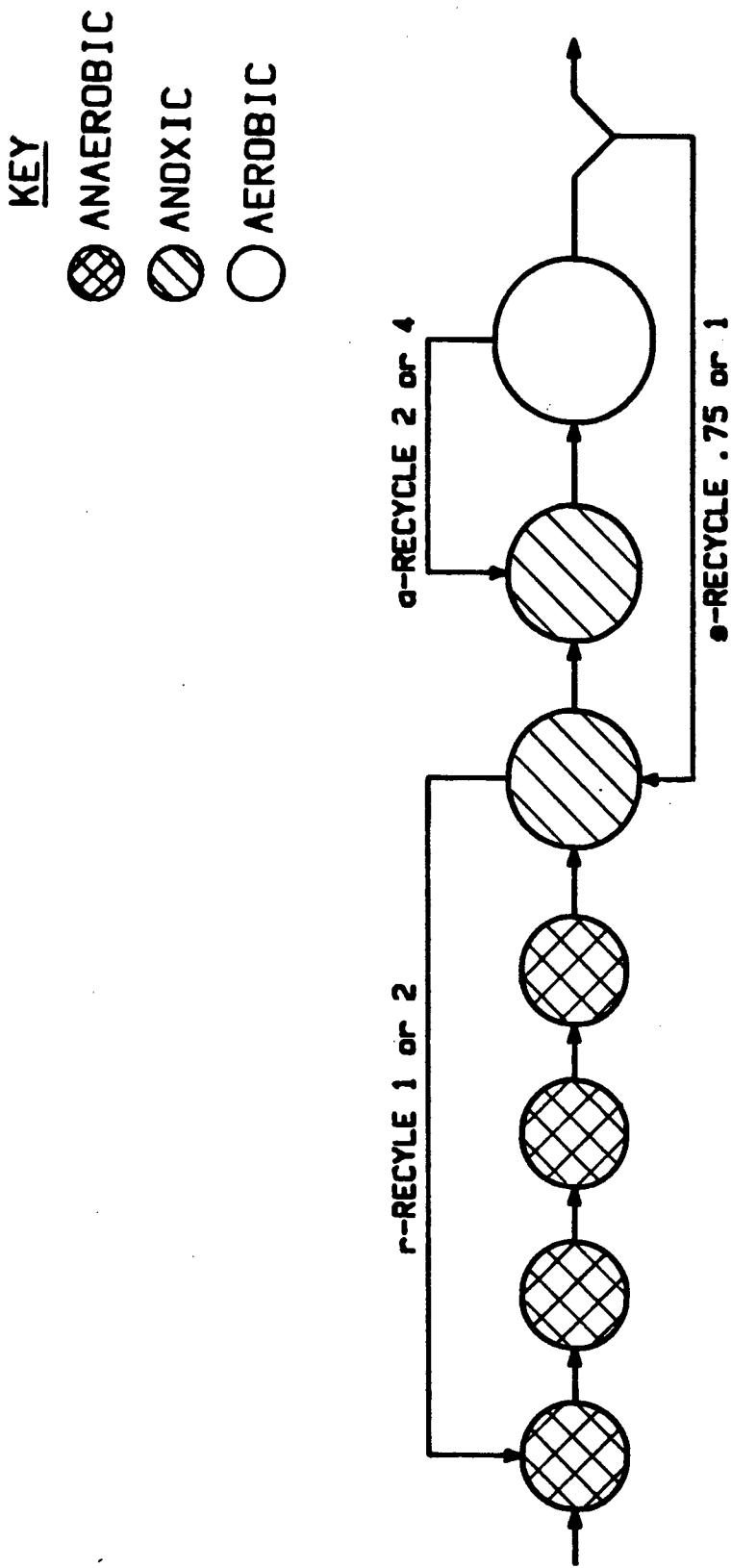


Fig 3.6(b): Schematic layout of the modified UCT system used in the experimental investigation.

cumulative release in the series of anaerobic reactors are shown in Figs 3.7(a) and 3.8(a). Release was simulated using the theory set out above, the constants K and C_{sp} found by curve fitting. Best overall fits were obtained for $K = 0,06/\text{day}$ and $C_{sp} = 0,5 \text{ mgP/mgCOD}$, the corresponding theoretical predictions being shown in Figs 3.7(b) and 3.8(b). Correlation between the predicted and observed cumulative release down these series, and for data from a 20 day sludge age unit, is shown in Figs 3.9(a and b) respectively.

The theory then was applied to the data from the batch and plug flow tests in Figs 3.3(a) and 3.4(a) respectively, see Figs 3.10(a and b). In the batch tests the constant K differed from 0,06, Fig 3.10(a), but that in the plug flow tests, Fig 3.10(b), remained unaltered at 0,06/day.

The reasonable correlation between observed and predicted data on P release over a wide range of process conditions and sewage characteristics lends strong support to the basic hypothesis and the quantitative theory developed from it.

5. IMPLICATIONS

By having a model to describe the kinetics of phosphorus release it is possible to investigate theoretically the effects of various parameters on P release. Parameters that influence P release can be subdivided into two groups, sewage characteristics and system characteristics.

Sewage characteristics (WRC Manual, 1984)

- (1) Influent COD, S_{ti}
- (2) Fraction of S_{ti} which is unbiodegradable particulate, i.e. $S_{up} = f_{S,up} S_{ti}$
- (3) Fraction of S_{ti} which is unbiodegradable soluble, i.e. $S_{us} = f_{S,us} S_{ti}$
- (4) Biodegradable influent COD i.e. $S_{bi} = S_{ti}(1 - f_{S,us} - f_{S,up})$
- (5) Fraction of S_{bi} which is readily biodegradable, i.e. $S_{bsi} = f_{S,bs} S_{bi}$

The above characteristics affect the release directly. Indirect effects occur due to nitrification and denitrification which may affect the nitrate discharged to the anaerobic reactor and hence S_{bsi} in accordance with Eq (3.2). The nitrate effect in turn is dependent upon the denitrification design of the plant and the TKN/COD ratio. Temperature also has an influence, but this was not addressed in the investigation.

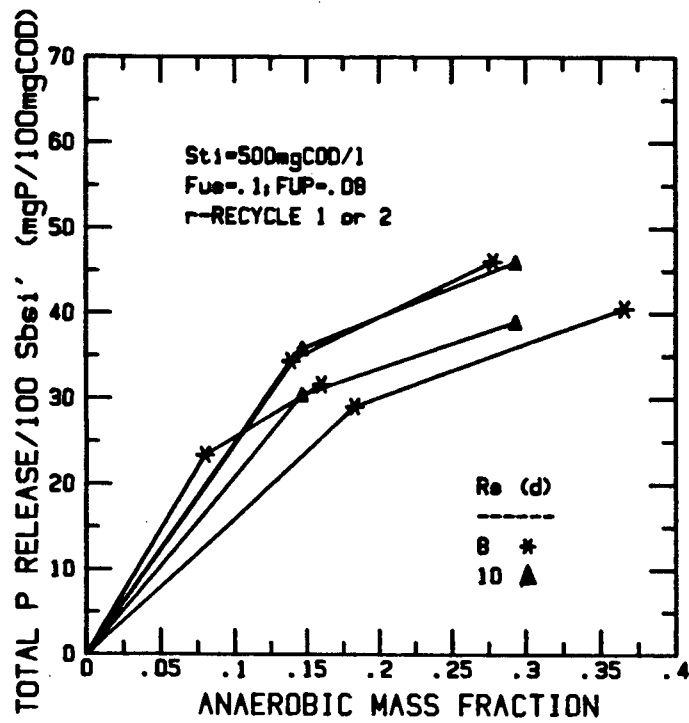


Fig 3.7(a): Observed cumulative P release in two-in-series anaerobic reactor of a modified UCT system with unsettled municipal wastewater as influent.

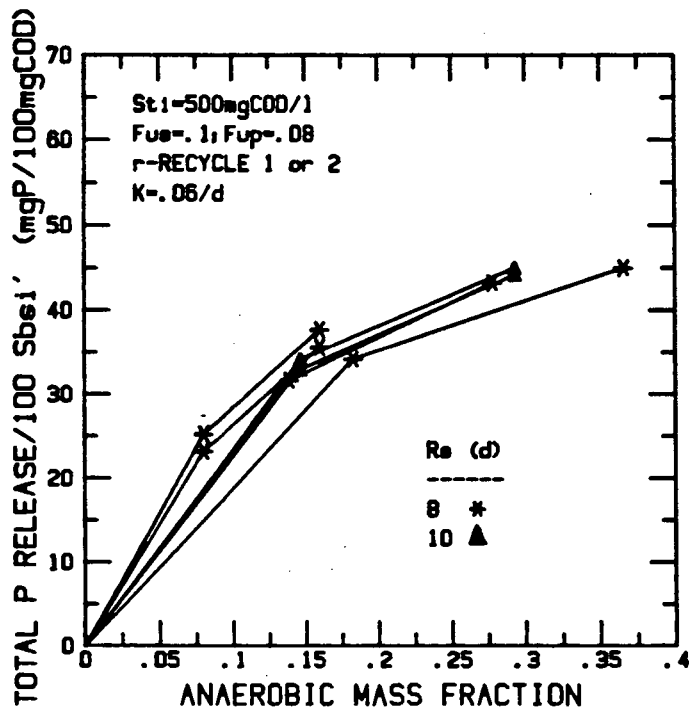


Fig 3.7(b): Simulated cumulative P release for systems in Fig 3.7(a).

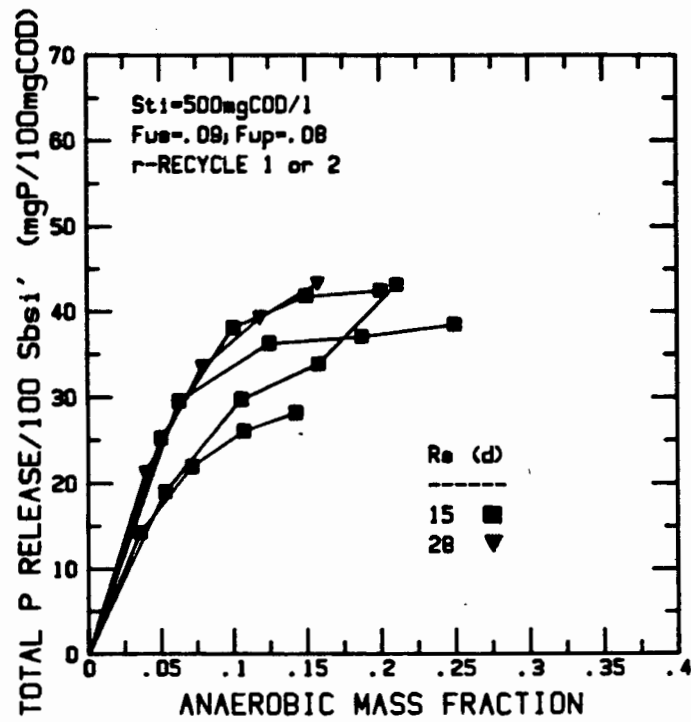


Fig 3.8(a): Observed cumulative P release in four-in-series anaerobic reactor of a modified UCT system with unsettled municipal wastewater as influent.

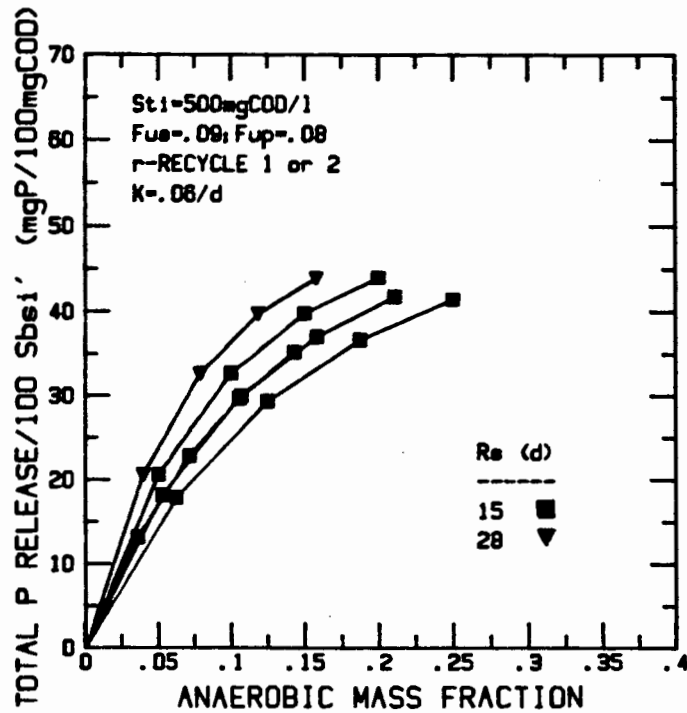


Fig 3.8(b): Simulated cumulative P release for systems in Fig 3.8(a).

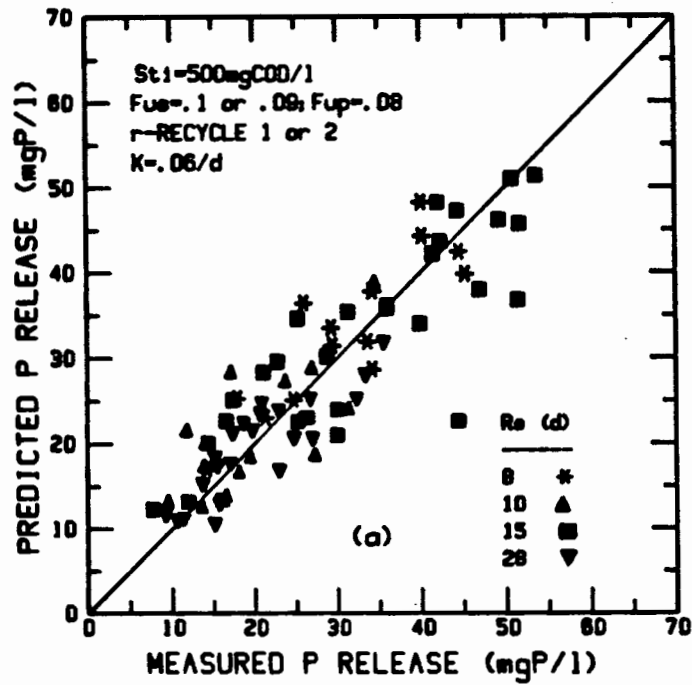


Fig 3.9(a):

Predicted versus measured cumulative P release down series of anaerobic reactors; data from Figs 3.7(a) and 3.8(a).

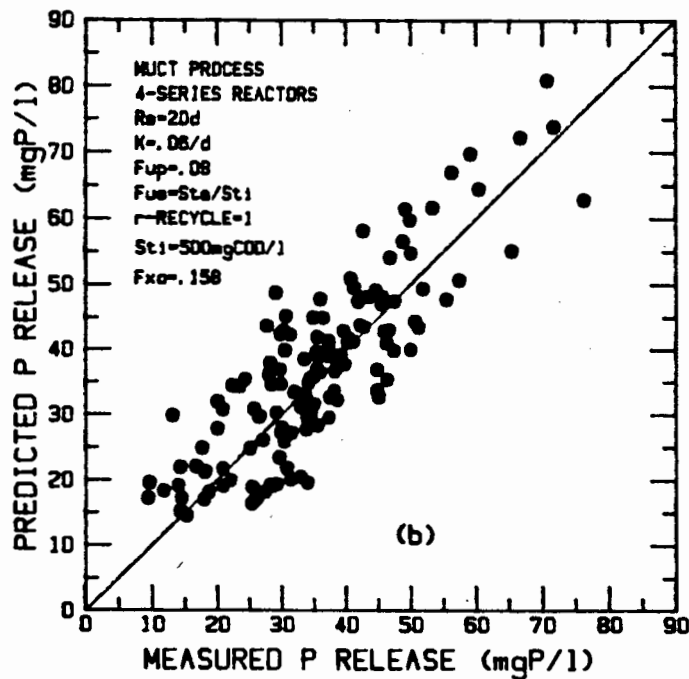
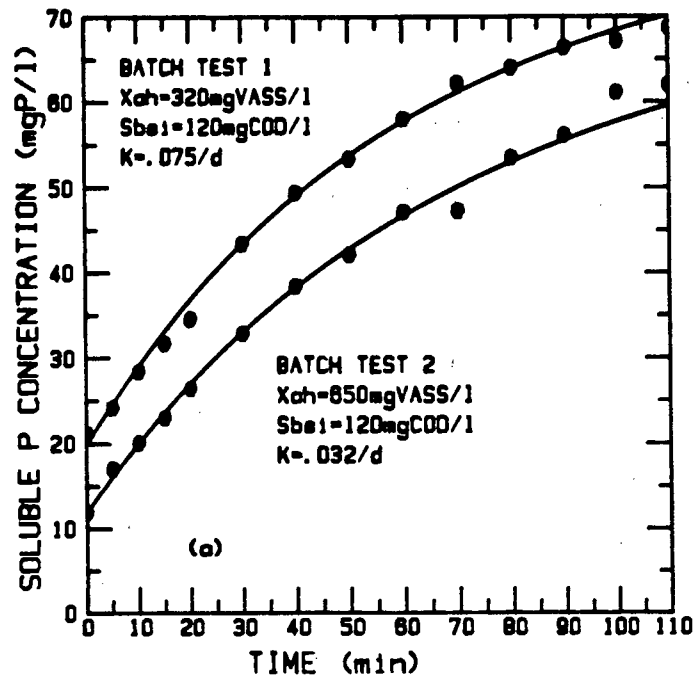
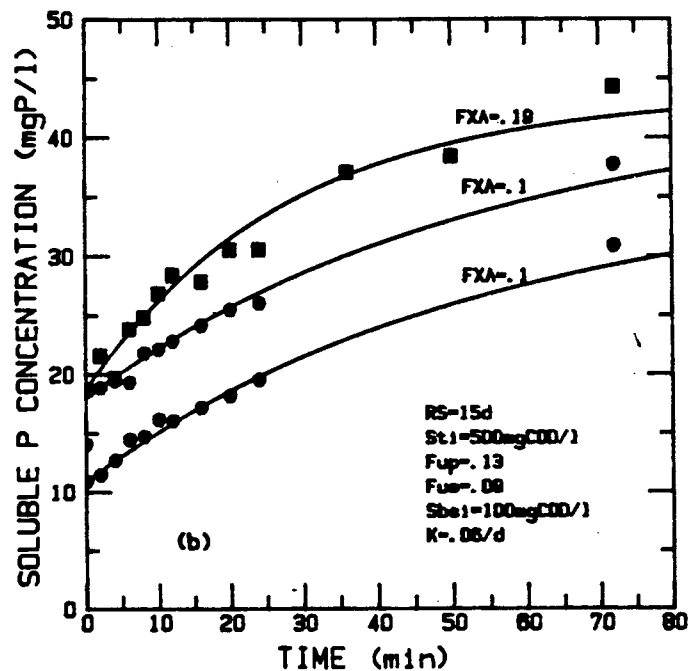


Fig 3.9(b):

Predicted versus measured cumulative P release down series of anaerobic reactors; data from 20 days sludge age system.

**Fig 3.10(a):**

Predicted phosphorus concentration profiles using kinetic theory for batch test data in Fig 3.3.

**Fig 3.10(b):**

Predicted phosphorus concentration profiles using kinetic theory for plug flow data in Fig 3.4 (plus other data sets).

System characteristics

- (1) System sludge age, R_s , = (mass of sludge in system)/(mass wasted per day)
- (2) The r-recycle in the UCT type system
- (3) The anaerobic mass fraction defined by (mass of sludge in anaerobic zone)/(total mass of sludge in system)
- (4) Series or single anaerobic reactor configuration.

Equations (3.9 and 3.11) were reformulated in terms of the anaerobic nominal hydraulic retention time instead of f_{xa} . A comparison of the predictions from these respective equations indicated that the f_{xa} is the superior parameter in terms of which to evaluate the effects of the various parameters above, also, that the parameter P release per S_{bsi} is convenient in expressing the release predicted.

With regard to the sewage characteristics, a 'normal' unsettled municipal wastewater in South Africa would have $f_{S,up} = 0,13$, $f_{S,us} = 0,07$ and $f_{S,bs} = 0,24$; settled wastewater has $f_{S,up} = 0,04$, $f_{S,us} = 0,10$ and $f_{S,bs} = 0,33$. In countries where garbage grinding is permitted preliminary estimates give very much higher values for $f_{S,up}$ of approximately 0,23; no data is available for these wastewaters when settled.

Accepting the characteristics for an unsettled wastewater from a South African municipal source and assuming that no nitrate enters the anaerobic reactor i.e. $S'_{bsi} = S_{bsi}$, also that $r = 1$, the release/100 mgS'_{bsi} versus f_{xa} is shown in Fig 3.11(a) for a single anaerobic reactor with S_{ti} of 250, 500 and 1000 $mgCOD/l$ and R_s of 10, 15, 20, 25 and 30 days. From Fig 3.11(a) R_s has a relatively minor effect on P release. The reason for this is that for $R_s > 10$, $MX_{B,H}/MS_{bi}$ remains relatively constant; for $R_s < 10$, the $MX_{B,H}/MS_{bi}$ declines, adversely affecting the conversion of S_{bsi} , and hence P release. S_{ti} has a marked effect on the release characteristics. The higher the S_{ti} the higher the P release per 100 S'_{bsi} for a fixed anaerobic mass fraction. This implies that the higher the S_{ti} the lower the anaerobic mass fraction to obtain the same release per S'_{bsi} .

Effect of subdividing the anaerobic reactor is shown in Fig 3.11(b). The plot is similar to that in Fig 3.11(a) but with the anaerobic mass subdivided into a series configuration of four. Comparing the release behaviour between Figs 3.11(a) and 3.11(b), series operation significantly improves the P release per 100 S'_{bsi} particularly

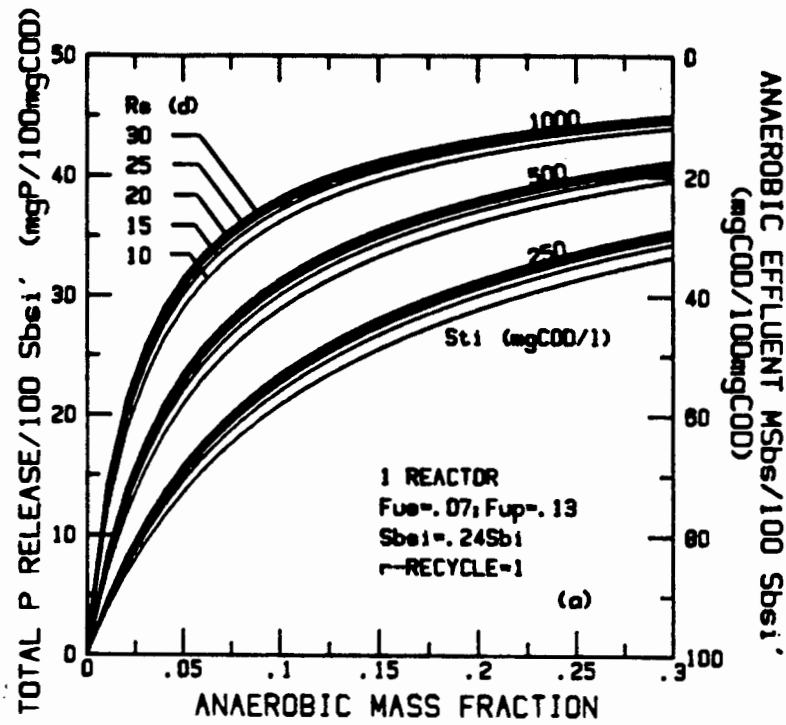


Fig 3.11(a):

Predictions of total anaerobic phosphorus release for a single anaerobic reactor system with influent COD's and system parameters shown.

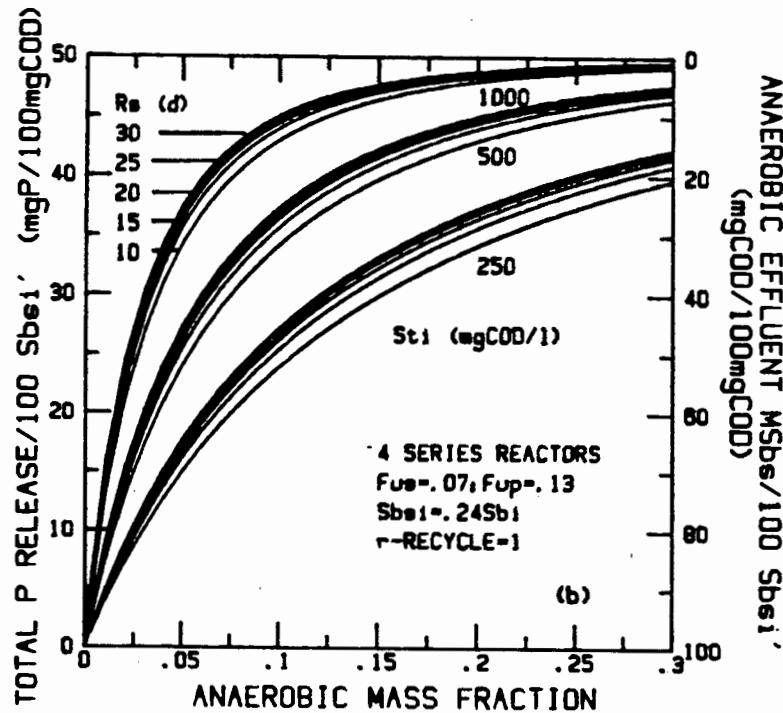


Fig 3.11(b):

Predictions of total anaerobic phosphorus release for a four-in-series anaerobic reactor system with influent COD's and system parameters shown.

at higher f_{xa} , or conversely, a lower anaerobic mass fraction is needed in series operations to give the same release as in a single reactor. A comparison between single, two-in-series and four-in-series systems (not shown) indicated that the main improvement is from single to two-in-series systems.

With regard to the effect of the r -recycle ratio, in Fig 3.12 the release in a single reactor system for $r = 2$ is shown. Comparing Figs 3.11(a) and 3.12, $r = 1$ is superior to $r = 2$. However in the UCT process the lower recycle requires a larger anaerobic *volume* to give the same f_{xa} as the higher recycle (see Appendix B or WRC Manual, 1984).

The effect of the S_{bsi}/S_{bi} ratio is indicated in Fig 3.13' for a single and a two-in-series anaerobic reactor system, $R_s = 15d$, and f_{xa} of 0,05, 0,10, 0,15, 0,20 and 0,30; $S_{ti} = 250$ and $S_{bi} = 200$ mgCOD/ ℓ . S_{bsi} is varied from zero up to S_{bi} , the S_{bpi} varying correspondingly from S_{bi} to zero, ($S_{bpi} + S_{bsi} = S_{bi}$). For this sewage S_{bsi} normally was approximately 50 mgCOD/ ℓ , clearly if the S_{bsi} can be increased the P release will increase almost linearly for any selected f_{xa} . However the increase in P release has a decreasing tendency with increase in S_{bsi}/S_{ti} so that even if the $S_{bsi}/S_{ti} = 200/250$ (i.e. $S_{bsi}/S_{bi} = 1$) the release obtained for $f_{xa} = 0,15$ would be only 43 mgP/ ℓ influent flow. The reason for the low release is that in this case $S_{bsi} = S_{bi}$, so that a large fraction of S_{bsi} is utilized to generate non-polyP heterotrophic mass. Where S_{bsi}/S_{bi} is relatively low, approximately 0,24 in South African municipal waste flows, considerable improvement in P release can be obtained by augmenting the S_{bsi} fraction, for example, by acid fermentation of the underflow from the primary settling tank and adding the fermented material to the inflow. However the kinetics of P release for these fermented wastes will change because the S_{bs} is already in the short chain fatty acid form and consequently does not need conversion to the short chain fatty acid form in the anaerobic reactor as required for a 'normal' S_{bsi} . This would not allow reduction of f_{xa} as the unfermented S_{bsi} still requires to be modified to short chain fatty acids.

6. P UPTAKE

Although this chapter has been concerned principally with P release, it was convenient in each of the steady state system tests to proceed further and obtain information also on P uptake. When batches of mixed liquor were taken from the aerobic zone of the UCT systems, further aeration produced virtually no additional P uptake; therefore it was accepted that the uptake reaction was virtually complete and that the aerated mass fraction ($\pm 50\%$) was adequate. It was of interest,

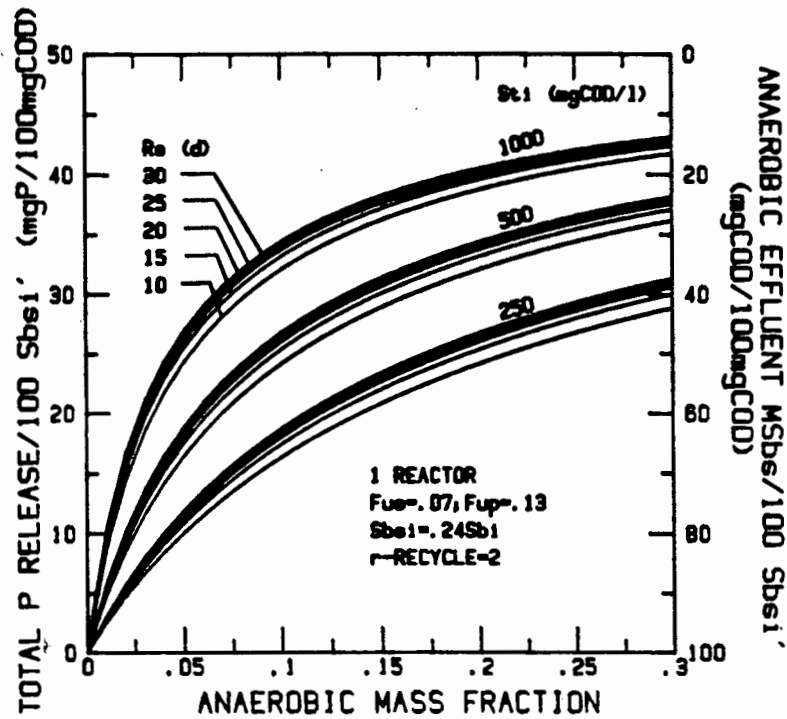


Fig 3.12: Predictions of total anaerobic phosphorus release for the influent COD's and system parameters shown, with a single anaerobic reactor and r-recycle of 2 (cf Fig 3.11(a) for $r = 1$).

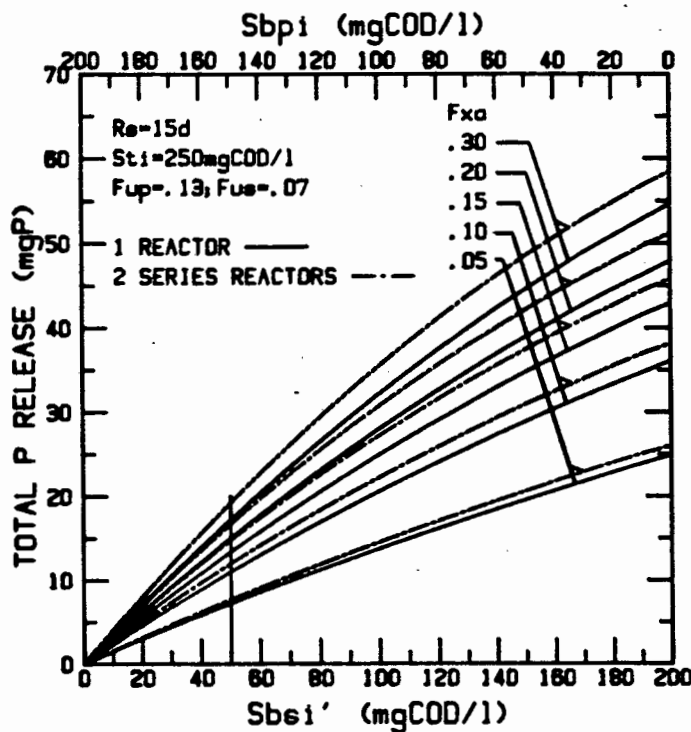


Fig 3.13: Effect of the magnitude of S'_{bsi} on anaerobic phosphorus release for a fixed total influent COD and system sludge age for selected anaerobic mass fractions.

therefore, to see if there is a relationship between the observed P release and observed P uptake. Plots of the daily P release versus P uptake for four of the sludge ages tested are shown in Figs 3.14(a,b,c and d). All the plots show a close linear relationship between P release and uptake, with the slopes having nearly the same magnitude irrespective of sludge age. That the slopes all should have nearly the same magnitude would indicate that the polyP organisms have endogenous mass loss characteristics very different from those observed for non-polyP organism masses - the specific endogenous mass loss rate of the polyP organism mass appears to be virtually zero, compared to a specific endogenous mass loss rate of 0,24 mgVASS/mgVASS/d for 'normal' heterotrophic organism masses in activated sludge systems (Marais and Ekama, 1976).

Accepting the observed linear relationship and postulating that the P value of the intercept on the uptake axis is that for the basic metabolic P requirements of the total organism mass, the relationship in Figs 3.14(a,b,c and d) can be modelled as

$$P(\text{uptake}) = a' P(\text{release}) + P(\text{metabolic}) \text{mgP}/\ell \quad (3.12)$$

or

$$P(\text{removal}) = P(\text{uptake}) - P(\text{release}) \quad (3.13)$$

$$= (a'-1) P(\text{release}) + P(\text{metabolic}) \quad (3.14)$$

Now $P(\text{metabolic})$ accounts for the normal metabolic P requirement wasted daily in the sludge. From trial and error it would appear that if the metabolic P content of the sludge is 3 percent with regard to VSS, then in Eq (3.12)

$$P(\text{metabolic}) = 0,03 MX_v / (Q \cdot R_s) \text{ mgP}/\ell \text{ influent} \quad (3.15)$$

where MX_v = mass of VSS in the system (Marais and Ekama, 1976).

The statistical fits for a' give values ranging from 1,145 to 1,198, see Figs 3.14(a,b,c and d). These values for a' appear to have only a slight decreasing tendency as R_s increases. Although this observation must be taken as tentative it would support the conclusion earlier that the endogenous mass loss of the polyP organisms is small.

Although it has been possible to present a formulation for the P uptake, this relationship is empirical. Whereas in the P release model the polyP organism mass was not implicated directly because the release rate is controlled by the S_{bs} modifications mediated by the non-polyP heterotrophs, excess P uptake will be

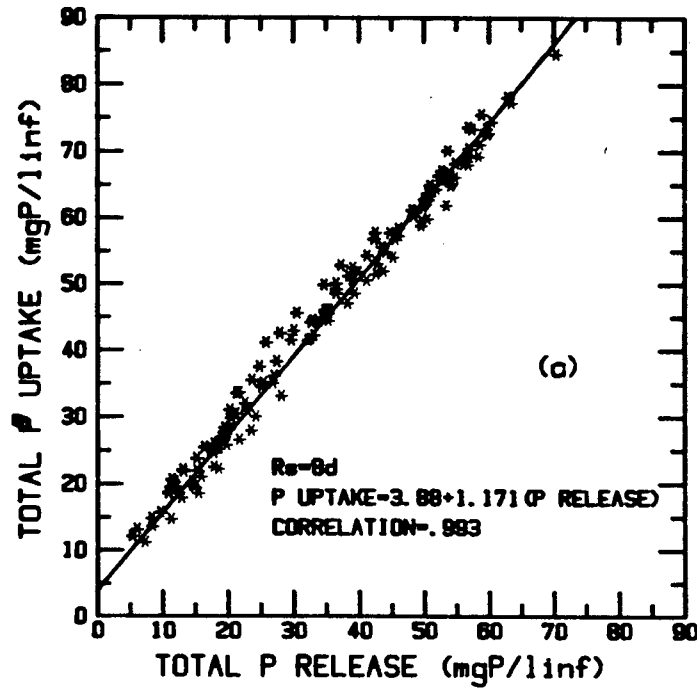


Fig 3.14(a):

Plot of total P uptake versus P release data pairs observed in this investigation for systems operated at sludge age of 8 days.

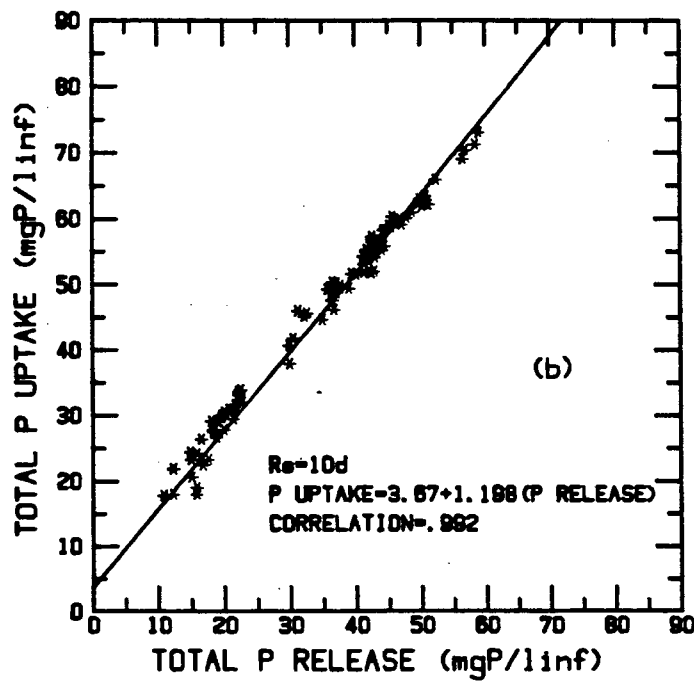


Fig 3.14(b):

Plot of total P uptake versus P release data pairs observed in this investigation for systems operated at sludge age of 10 days.

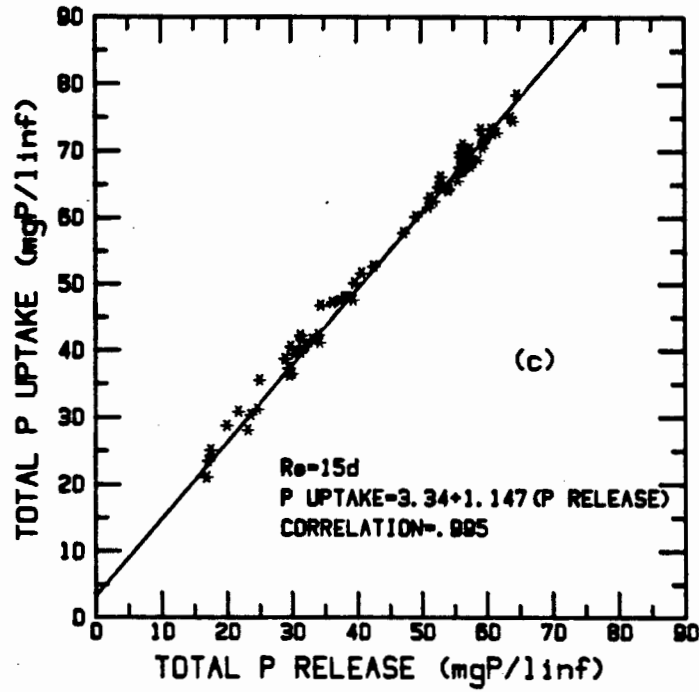


Fig 3.14(c):

Plot of total P uptake versus P release data pairs observed in this investigation for systems operated at sludge age of 15 days.

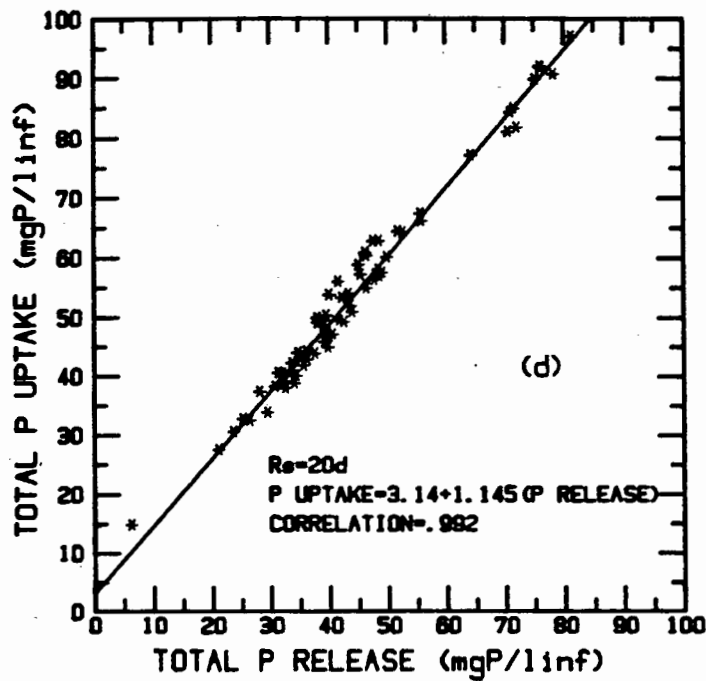


Fig 3.14(d):

Plot of total P uptake versus P release data pairs observed in this investigation for systems operated at sludge age of 20 days.

controlled almost completely by the action of the polyP organism mass. Hence, P uptake cannot be modelled effectively unless information on the polyP organisms, particularly on their metabolic and kinetic behaviour, are known. It is not possible to obtain this information from the normal mixed culture activated sludge systems for reason that the response of the non-polyP heterotrophs either obscure or swamp out the response of the polyP organisms. The only way in which the characteristics of these organisms are likely to be described effectively, would be to develop pure, or relatively pure, cultures under environmental conditions similar to those in the mixed cultures of biological excess P removal systems. The difficulties in this regard are that very little is known of the microbiology and biochemistry of these organisms.

7. CONCLUSIONS

The following conclusions can be made with regard to anaerobic P release in biological excess P removal systems:

- (1) In the anaerobic reactor readily biodegradable COD from the influent is converted via the glycolytic (Embden-Meyerhof) pathway into short chain fatty acid forms by non-polyP heterotrophs. The rate of conversion appears to be first order with respect to the active non-polyP organism concentration in the anaerobic zone ($X_{B,Hn}$) and the readily biodegradable COD concentration (S_{bs}), i.e. $dS_{bs}/dt = K X_{B,Hn} S_{bs}$.
- (2) In the anaerobic reactor the short chain fatty acids are sequestered by the polyP organisms; energy for sequestration is obtained from hydrolysis of polyP chains accumulated in the organism. Hydrolysis results in the release of P to the liquid phase; the mass of P release (ΔP) appears to be proportional to the mass of COD sequestered (ΔS_{bs}) in the approximate ratio $\Delta P:\Delta S_{bs} = 1:2 \text{ mg(PO}_4\text{-P)/mgCOD}$.
- (3) The rate of sequestration appears to be faster than the rate of conversion; thus the conversion step is rate limiting for sequestration, and hence P release.
- (4) Application of the P release kinetics to biological excess P removal systems shows that the fraction of the total sludge in the anaerobic reactor is an important parameter in assessing P release behaviour. Because the reaction is first order series subdivision of an anaerobic reactor increases P release.

- (5) For a selected reactor configuration and fixed sewage characteristics the P release per unit of influent S_{bs} increases with increasing total influent COD.

With regard to excess P uptake one may conclude as follows:

- (1) Experimental observation indicates a linear relationship between the mass of P release in the anaerobic reactor and the mass of P uptake in the anoxic/aerobic part of the process.
- (2) The slope of the linear relationship is virtually constant irrespective of sludge age; this indicates that the specific endogenous mass loss rate of the polyP organisms is very small.
- (3) It does not appear to be possible to determine the characteristics of the polyP organisms or the processes involved in P uptake from the mixed cultures. It would appear that the polyP organisms responses need to be isolated. In this regard it seems that three aspects need attention:
 - (i) **Microbiological:** Information on the characteristics and presence of polyP organisms in mixed cultures of activated sludge systems under different environmental conditions, in aerobic and anaerobic/anoxic/aerobic systems.
 - (ii) **Biochemical:** Development of a biochemical model of polyP organism behaviour. Such a model will identify the biochemical processes involved, give information on stimulation, inhibition and regulation of these and, assist in identifying the conditions required to develop pure, or relatively pure, cultures.
 - (iii) **Population development:** It is essential that a pure or relatively pure culture of polyP organisms be developed under the same environmental conditions as in the biological excess P removal system. Such cultures should provide the material for identification and selection of the processes and compounds essential for macroscopic descriptions of biological excess P removal in activated sludge systems, and for obtaining data on the kinetics of the processes.

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CHAPTER 4

SELECTED CHARACTERISTICS OF *Acinetobacter* spp.

1. INTRODUCTION

In the previous chapter it was concluded that modelling of biological excess P uptake requires, *inter alia*, information on the polyP organisms that are involved in biological excess P removal in activated sludge systems and on their characteristics. One group of polyP organisms reputed to be important in biological excess P removal is the genus *Acinetobacter* (Fuhs and Chen, 1975). The presence of organisms from this genus in anaerobic/aerobic and anaerobic/anoxic/aerobic systems exhibiting biological excess P removal is widely accepted (*inter alia* Buchan, 1983; Lötter and Murphy, 1985). Accordingly it was decided to investigate selected characteristics of these organisms and their interaction with different conditions, from a review of the literature and experimental observations.

This chapter records the investigation.

2. CHARACTERISTICS OF *Acinetobacter* spp.

Acinetobacter spp. grow well on simple mineral media containing a single carbon compound, this compound serving both as a carbon and as an energy source for anabolic processes (Juni, 1978). They can be cultured equally well on liquid and on solid media (Warskow and Juni, 1972; Henriksen, 1973; Juni, 1978). The ability of *Acinetobacter* spp. to use oxygen as external electron acceptor is well documented (Juni, 1978); more recently it has been shown that some strains also can utilize nitrate as external electron acceptor when oxygen is absent (Henriksen, 1976, Lötter, 1985; van Groenestijn and Deinema, 1985). Of particular interest is carbon metabolism in *Acinetobacter* spp.: All *Acinetobacter* strains grow aerobically on acetate (Baumann *et al.*, 1968); some strains can utilize glucose but do so exclusively via the Entner-Doudoroff pathway (McDonald and Juni, 1973), a pathway inoperative under anaerobic conditions¹. Furthermore, none of the strains appear to possess the glycolytic (Embden-Meyerhof) pathway (Juni, 1978). In consequence glucose, and similar compounds, can be metabolized by strains possessing the Entner-Doudoroff pathway only where an external electron acceptor (nitrate or oxygen) is available; strains not possessing the Entner-Doudoroff pathway cannot metabolize such compounds at all. The tricarboxylic acid (Krebs) cycle functions in

¹By anaerobic is meant the absence of both nitrate and oxygen, and no input of these.

Acinetobacter spp., as evidenced by the detection of the key enzymes of that cycle in cell-free extracts (Juni, 1978). Key enzymes of the anaplerotic glyoxylate cycle also have been identified; in fact these two cycles appear to function simultaneously in *Acinetobacter* spp. (Sturm *et al.*, 1970; Juni, 1978). Poly- β -hydroxybutyrate (PHB) accumulation has been demonstrated also in *Acinetobacter* spp. (Lawson and Tonhazy, 1980). Furthermore, these organisms are capable of storing phosphorus as polyphosphate in metachromatic granules called volutins (Fuhs and Chen, 1975; Deinema *et al.*, 1980; Buchan, 1983).

2.1 Application to aerobic and anaerobic/aerobic systems

Having described above the known key characteristics of *Acinetobacter* spp., it is possible to speculate on the interaction between these characteristics and the environment, in (1) completely aerobic and (2) anaerobic/aerobic systems.

- (1) Completely aerobic systems: *Acinetobacter* strains with an Entner-Doudoroff pathway can utilize sugars, e.g. glucose, under aerobic and anoxic conditions and thus should grow in competition with other heterotrophs in anoxic/aerobic or completely aerobic systems. *Acinetobacter* strains that do not possess the Entner-Doudoroff pathway can utilize only short chain fatty acids, e.g. acetate, and thus would be at a severe disadvantage as many wastewaters contain relatively small fractions of such substrates (Chapter 3, section 2.1; Pitman and Lotter, 1986). In consequence *Acinetobacter* strains with an Entner-Doudoroff pathway can be expected to be present in aerobic systems receiving municipal wastewaters. Although the presence of *Acinetobacter* spp. has been noted in anoxic/aerobic systems not removing P in excess (Cloete, 1984; Lötter and Murphy, 1985), the literature records no reference to their presence in completely aerobic activated sludge systems.
- (2) Anaerobic/aerobic systems: When an anaerobic phase is introduced into an activated sludge system there is no information on how "aerobic" *Acinetobacter* strains, i.e. with an Entner-Doudoroff pathway, will react. However, what one can say is that, if short chain acids are added to the anaerobic phase, *Acinetobacter* which have a polyphosphate pool will utilise the polyphosphate as an energy source to take up the acids and store these as polyhydroxybutyrate (PHB) (Rensink, 1981); subsequently in anoxic and aerobic zones the stored PHB is utilized for growth and for storage of phosphorus as polyphosphate (see Chapter 5 for a detailed biochemical model). If a substrate like glucose is added to the anaerobic phase, then

facultative organisms, capable of fermentation, will break down the glucose to short chain fatty acids, as shown in Chapter 3; the acids are discharged to the medium and become available for sequestration by *Acinetobacter* with a polyphosphate pool. *Acinetobacter* spp. which lack a polyphosphate pool will not be able to sequester substrate in the anaerobic phase. Hence under anaerobic/aerobic sequencing, the conditions will favour *Acinetobacter* spp. possessing a polyphosphate pool, irrespective of whether these possess an Entner-Doudoroff pathway, or not. Numerous references in the literature are made to the presence of *Acinetobacter* spp. with a polyP pool in anaerobic/aerobic sequencing activated sludge systems (*inter alia* Fuhs and Chen, 1975; Buchan, 1983).

From the above speculations, if the aerobic system is changed to an anaerobic/aerobic one, then one of two behaviour options exist – either the "aerobic" strain (with the Entner-Doudoroff pathway) has the propensity to store polyphosphate, invokes it, and then sequesters acetate under anaerobic conditions, or, a new strain of *Acinetobacter* able to store polyphosphate develops, perhaps without the Entner-Doudoroff pathway. The literature is sparse on providing guidance on this matter:

Fuhs and Chen (1975), when studying the nutritional spectrum of an *Acinetobacter* strain isolated from an anaerobic/aerobic excess phosphorus removal system, found that the isolate grew on short chain fatty acids but could not use glucose as substrate, which implies that the isolate did not possess the Entner-Doudoroff pathway. Thus one might conclude that *Acinetobacter* strains that may be found in completely aerobic systems differ from the strains found in anaerobic/aerobic systems in that "aerobic" strains possess the Entner-Doudoroff pathway whereas "anaerobic/aerobic" strains do not. The work of Rensink (1981) similarly does not supply an answer: Rensink operated a twelve-in-series reactor aerobic activated sludge plant which did not exhibit phosphorus release, uptake or excess phosphorus removal. The first six reactors in the series were converted to operate without aeration. Immediately after the change, on batch augmenting the feed with acetate, virtually no acetate disappeared in passing through the first six anaerobic reactors. Over a six-week period with similar batch augmentation tests, the rate of disappearance of the acetate increased, until finally, virtually all the acetate disappeared in the first two reactors. Concomitant with the acetate disappearance, phosphorus release increased in the anaerobic reactors, as did the phosphorus uptake in the aerobic reactors and

phosphorus removal by the system. Clearly the phosphorus release, uptake and removal developed over a matter of weeks. Assuming that, during the initial aerobic operation, *Acinetobacter* spp. were present, it is not clear whether the long period for the development of P removal was required for a new *Acinetobacter* strain to become established in the system, this new strain possessing the propensity for polyP accumulation, or, whether the strains present in the aerobic system required this long period to invoke a latent propensity for polyP accumulation via development of the appropriate enzyme systems resulting in a progressive increase in polyP and PHB.

From the speculations above two questions are prominent;

- (1) can *Acinetobacter* spp. be present in completely aerobic systems and, if so,
- (2) are the *Acinetobacter* strains or species in the aerobic systems the same as those in anaerobic/aerobic systems which give excess P removal?

To find answers to the questions, it was decided to isolate *Acinetobacter* strains from an anaerobic/anoxic/aerobic system exhibiting excess P removal and from two aerobic systems not exhibiting excess P removal. The *Acinetobacter* strains isolated were to be used to determine (1) the relative proportion of *Acinetobacter* spp. in each system; the strains then were to be tested for the ability to (2) reduce nitrate, (3) accumulate polyphosphate and polyhydroxybutyrate and, (4) utilize acetate and glucose as substrate. By comparing of the responses of the *Acinetobacter* isolates from the different systems it should be possible to come to some conclusion.

3. MATERIALS AND METHODS

Three laboratory scale activated sludge systems were operated:

- (1) A system comprising a single completely mixed aerobic reactor (15ℓ).
- (2) A system comprising two-in-series completely mixed aerobic reactors, the first a "selector" (0,3ℓ), the second the main reactor (14,7ℓ); both systems received settled municipal wastewater.
- (3) A modified Bardenpho system receiving a mixture of unsettled municipal wastewater and acetate.

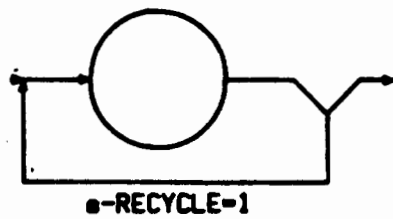
The configuration details are given in Fig 4.1 and the design/operational details in Table 4.1. Note that all systems were operated at 20 days sludge age. The single reactor system served as a control. The aerobic 'selector' system and the modified Bardenpho system merit the following additional comments.

The 'selector' reactor, with a volume of about 2 percent of the volume of the main reactor, is a configuration that has been devised to control and inhibit the growth of certain filamentous organisms that cause bulking. According to Jenkins *et al.* (1985) and Still *et al.* (1986), the 'selector' provides a region of high substrate/active mass ratio and appears to select for organisms with high maximum specific growth rates — maximum specific growth rates in the selector system have been measured to be 2,0 to 2,5 times those in the single aerobic reactor system (Still *et al.*, 1986). The relative volumetric size of the 'selector' is determined from the requirement that all the readily biodegradable COD in the influent will be utilized in the 'selector'.

In the modified Bardenpho system the objective was to study the increase in P removal on supplementing the influent with acetate; acetate can be directly 'utilized' by *Acinetobacter* spp., by sequestration in the anaerobic reactor, so that the increased substrate available to these organisms could be expected to favour their proliferation and to give rise to increased P removal. Indeed P removal did increase approximately proportionately to the increase in acetate fraction of the influent. Details of the development and response of this system are given in Chapter 6.

Mixed liquor samples from the aerobic zone of the Bardenpho system which exhibited excess P removal and from the two completely aerobic systems both not exhibiting excess P removal (see Table 4.1), were subjected to the isolation and identification procedure described by Lötter and Murphy (1985). Twenty-five *Acinetobacter* isolates from each system were retained for the following studies:

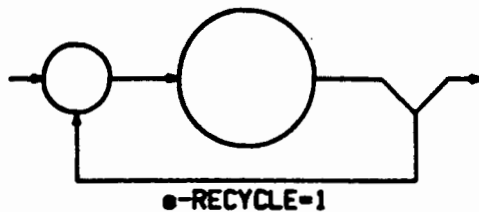
- (1) The nitrate reducing propensity of each isolate was tested by growth in nitrate agar and subsequent determination of nitrogen and nitrite (Difco, 1957).
- (2) Polyhydroxybutyrate (PHB) accumulation by the *Acinetobacter* isolates was determined by growth on nutrient agar augmented with β -hydroxybutyrate (Bovre *et al.*, 1972) and then examining cells microscopically for PHB inclusions after Sudan Black staining, as described by Gurr (1973).



(a): Single completely mixed aerobic reactor system.

SYSTEM CHARACTERISTICS

$R_s = 20d$ $Q = 15\ell/d$
 $St_i = 350\text{mgCOD}/\ell$
 $VOLUME = 15\ell$



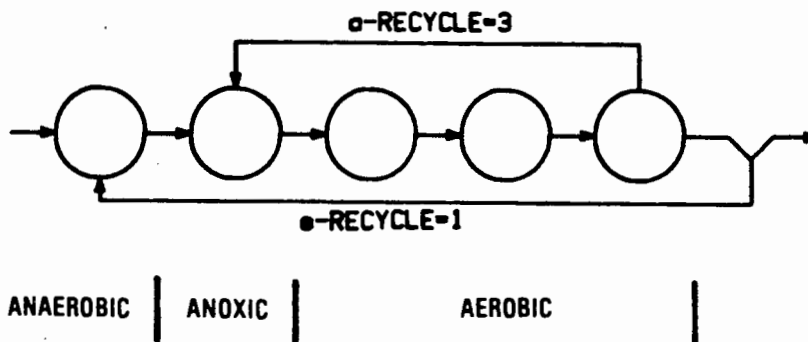
(b): Completely mixed aerobic system with selector.

SYSTEM CHARACTERISTICS

$R_s = 20d$ $Q = 15\ell/d$
 $St_i = 350\text{mgCOD}/\ell$

VOLUMES

$SELECTOR = 0.3\ell$
 $MAIN AEROBIC = 14.7\ell$

**SYSTEM CHARACTERISTICS**

$R_s = 20d$ $Q = 15\ell/d$
 $F_{xa} = .15$ $V_t = 10\ell$
 $St_i = 500\text{mgCOD}/\ell$

VOLUMES

$ANAEROBIC = 1.5\ell$
 $ANOXIC = 1.0\ell$
 $AEROBIC 1 = 1.5\ell$
 $AEROBIC 2 = 3.0\ell$
 $AEROBIC 3 = 3.0\ell$

(c): Modified Bardenpho system.

Fig 4.1:

Configurational details of the three laboratory scale systems operated to produce mixed liquor for the investigation.

Table 4.1: Design/operational details of the three laboratory scale systems from which mix liquor samples were taken for isolation of *Acinetobacter* spp.

System parameter	Completely aerobic systems		Bardenpho system
	single reactor	with selector	
Sludge age (d)	20	20	20
Substrate	settled municipal wastewater	settled municipal wastewater	Raw municipal wastewater + acetate supplement
Influent COD (mgCOD/l)	350	350	200 wastewater 300 acetate
Influent TKN (mgN/l)	50	50	25
Flow rate (l/d)	15	15	15
P removal (mgP/l)	2	2	15

- (3) Growth and substrate utilization with acetate and glucose were determined as follows: Each isolate was grown in Fuhs and Chen (1975) nutrient medium. Solutions (250 mL) of the medium, containing only acetate or glucose as substrate at a theoretical COD of 4 000 mg/L, were inoculated with 1,0 mL of a cell suspension with an optical density of 1,0 ($\lambda = 520$) and incubated aerobically for five days at 20°C. Thereafter the cell suspensions were centrifuged in tared centrifuge tubes at 20 000 g. The supernatant was retained for the determination of COD by the dichromate oxidation method as described in Standard Methods (1985). The concentration of cells was determined by massing the dried cells.
- (4) Polyphosphate accumulation was determined by making a slide of each cell suspension after the incubation period above, and staining the slides with a Neisser stain (Lötter and Murphy, 1985).

4. RESULTS AND DISCUSSION

4.1 Presence of *Acinetobacter* spp.

The fractions of *Acinetobacter* spp. in the sludges are given in Table 4.2. Evidently the aerobic systems each developed an appreciable fraction of *Acinetobacter* spp.

4.2 Nitrate reduction

The results of the nitrate reducing capacity of *Acinetobacter* isolates are given in Table 4.3. It is evident from Table 4.3 that a number of *Acinetobacter* isolates were capable of nitrate reduction. This is in conformity with the findings of previous workers on the capacity for nitrate reduction by *Acinetobacter* spp. (Lötter, 1985; van Groenestijn and Deinema, 1985). Also it is evident that, in their capacity for nitrate reduction, there was little difference between isolates from the anaerobic/anoxic/aerobic and the aerobic systems. Thus the propensity for nitrate reduction did not appear to be induced by environmental conditions, that is, the inclusion of an anoxic zone in the activated sludge system did not augment the capacity for nitrate reduction in *Acinetobacter* spp. in the system. The majority of the *Acinetobacter* isolates able to reduce nitrate, reduced the nitrate to nitrite, only a minority of isolates reduced nitrate to nitrogen.

4.3 Polyphosphate and polyhydroxybutyrate accumulation

The slides of the isolates stained for the presence of polyphosphate and polyhydroxybutyrate were analysed as follows: For each isolate the slide was categorised by assigning to the slide a value of 0 to 4 according to the number of

cells containing inclusions as follows: If none of the cells contain inclusions — 0; about one quarter of the cells — 1; one half of the cells — 2; three quarters of the cells — 3; and all the cells — 4. For each system the number of isolates in each category was expressed as a percentage of the total number of isolates. These results are depicted graphically in Figs 4.2(a, b and c).

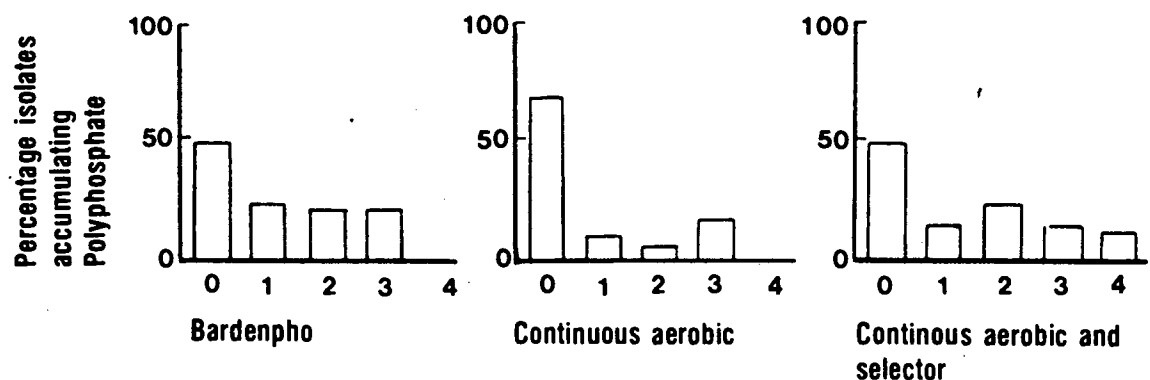
Referring to Figs 4.2 (a and b), polyphosphate accumulation occurred in *Acinetobacter* isolates from both aerobic systems and from the anaerobic/anoxic/aerobic systems, on both glucose and acetate substrates. Although the modified Bardenpho system favoured the growth of *Acinetobacter* spp., indicated by the fact that 90 percent of the organisms were *Acinetobacter* spp., the proportion of isolates from this system that exhibited polyP accumulation was not significantly higher than those from the aerobic systems. Indeed the modified Bardenpho isolates contained a higher proportion of isolates exhibiting zero presence of polyphosphates than the aerobic systems. It would appear that the *Acinetobacter* isolates from the completely aerobic systems, while not accumulating polyphosphate in the system, did in fact possess a similar propensity to accumulate polyphosphate (under the aerobic culture conditions) as isolates from the anaerobic/anoxic/aerobic system where *Acinetobacter* strains did accumulate polyphosphate. Thus the environment of the system from which the *Acinetobacter* was isolated, namely anaerobic/anoxic/aerobic or aerobic only, did not appear to have a significant effect in selecting for strains that have the propensity to accumulate polyphosphate, but rather that the anaerobic/aerobic system invoked a latent propensity in the organism for polyP accumulation.

With regard to storage of polyhydroxybutyrate, all the isolates, irrespective of their origin, appeared to exhibit similar propensities to accumulate polyhydroxybutyrate under the aerobic culture conditions [see Fig 4.2(c)] even though this accumulation has not been observed in completely aerobic activated sludge systems.

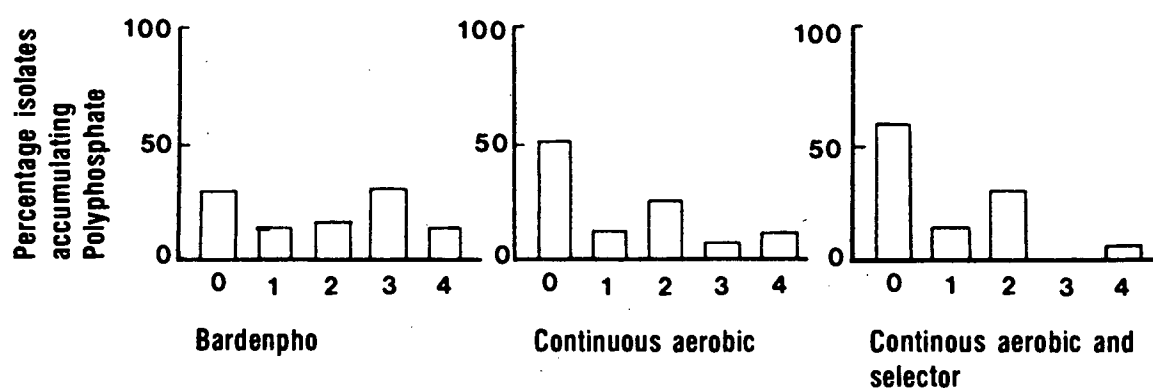
From the above it would appear that propensities to accumulate polyhydroxybutyrate and polyphosphate are inherent characteristics of some *Acinetobacter* strains regardless of the system from which they are isolated.

4.4 Glucose and acetate utilization

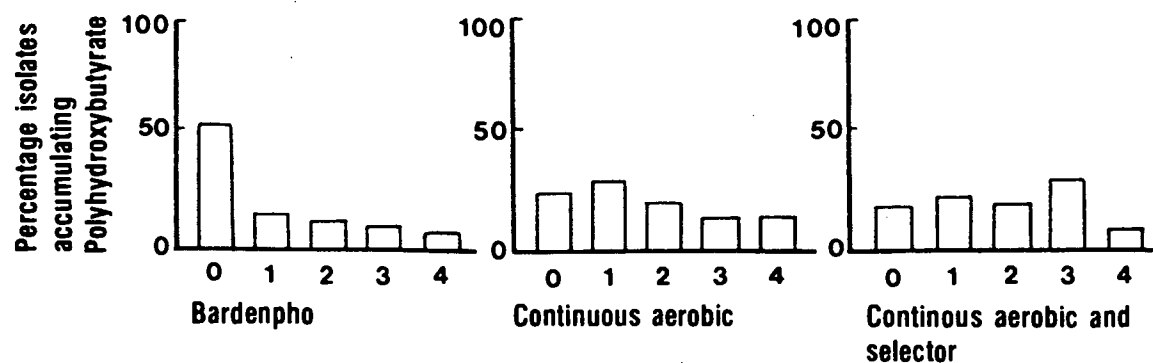
For each isolate the growth was measured over the 120 hour incubation period, on glucose and acetate respectively, and the mean growth calculated for the isolates from each system, see Table 4.4.



(a) Polyphosphate accumulation in acetate medium



(b) Polyphosphate accumulation in glucose medium

(c) Polyhydroxybutyrate accumulation on nutrient agar containing β -HO butyrate**Fig 4.2:**

Distribution of isolates showing accumulation of polyphosphate with acetate and glucose, and of polyhydroxybutyrate with β -HO butyrate.

Key:

- 0 - No cells contain inclusions
- 1 - One quarter of cells contain inclusions
- 2 - Half cells contain inclusions
- 3 - Three quarter cells contain inclusions
- 4 - All cells contain inclusions

Table 4.2: Percentages of total viable bacterial colonies that are *Acinetobacter* spp. in sludges of three systems.

System	Percentage <i>Acinetobacter</i> spp.
Completely mixed single aerobic reactor	40
Aerobic series with selector	60
Bardenpho	90

Table 4.3: Percentage of *Acinetobacter* isolates capable of nitrate reduction

Isolate source	Nitrate reduction to nitrite to nitrogen	
	Percentage of total (25 samples/system)	
Aerobic zone Bardenpho unit	32	6
Continuous aerobic unit	43	5
Continuous aerobic unit with selector	42	12

Table 4.4: Mean growth of isolates on acetate and glucose over 120 hrs.

Source of isolates	Mass of cells produced (mg) Acetate Glucose	
	(25 samples/system)	
Bardenpho aerobic zone	159	168
Continuous aerobic unit	146	186
Continuous aerobic unit with selector	188	198

These results indicate that isolates from all the systems could utilize acetate and glucose as substrate under aerobic conditions. Over the test period, from the measurement of soluble COD remaining after 120 hours, the substrate was practically depleted so that it was not possible to assess whether some systems produced isolates with higher growth rates than others. The data from Table 4.4 indicates that, contrary to the findings of Fuhs and Chen, *Acinetobacter* isolates from an anaerobic/anoxic/aerobic system can use glucose as substrate i.e. they possess the Entner-Doudoroff pathway. Furthermore, from Figs 4.2(a, b and c), under aerobic conditions, some of these isolates accumulated polyP both with glucose and with acetate as substrates, and exhibited an ability to accumulate PHB. In contrast, in aerobic activated sludge systems the *Acinetobacter* spp. accumulated neither polyP nor PHB. Hence it would appear that in the aerobic systems and in the aerobic cultures, the different conditions induced the organisms to respond in the different ways described above. The reasons for this behaviour will be discussed in Chapter 5.

5. CONCLUSIONS

The following conclusions can be drawn from the study:

- (1) *Acinetobacter* strains can be present in appreciable concentration in both anaerobic/anoxic/aerobic systems that exhibit excess P removal and completely aerobic systems that do not exhibit excess P removal.
- (2) There appear to be no significant differences in the *Acinetobacter* strains isolated from the anaerobic/anoxic/aerobic system and from the aerobic system despite the apparent different selective pressures created in the systems.
- (3) A number of the *Acinetobacter* strains isolated from anaerobic/anoxic/aerobic and from completely aerobic activated sludge systems can utilize glucose aerobically as substrate; such utilization most likely is via the Entner-Doudoroff pathway, a pathway which operates only with oxygen and/or nitrate present.
- (4) *Acinetobacter* strains isolated both from systems exhibiting excess phosphorus removal and from systems that do not, have the propensity to accumulate polyphosphate (polyP) and polyhydroxybutyrate (PHB) under aerobic culture conditions, with acetate and with glucose as substrate.

- (5) Imposing conditions conducive to excess phosphorus removal in a system (by anaerobic/aerobic sequencing) does not appear to stimulate new *Acinetobacter* strains, but rather to stimulate the polyphosphate and polyhydroxybutyrate accumulating propensities inherent in strains already present.

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CHAPTER 5

METABOLIC BEHAVIOUR OF *Acinetobacter* spp. IN BIOLOGICAL PHOSPHORUS REMOVAL - A BIOCHEMICAL MODEL

1. INTRODUCTION

Since the phenomenon of biological excess phosphorus removal in activated sludge systems first was observed (Srinath *et al.*, 1959), attempts have been made to elucidate the mechanisms governing it. Over the years various hypotheses and models have been proposed; each successive model has been influenced in some degree by the models preceding it, but often the factors on which a model conceptualization has been based are not clear. In this chapter the evolution of these models is traced briefly but critically. Then yet another model is proposed which, it is hoped, will resolve some of the difficulties that have become apparent with previous models.

The research papers that either directly or indirectly influenced the conceptualization of the respective models are extensive and it is not possible to include a critical evaluation of each; only those papers of major direct influence will be mentioned. For a detailed review of this topic, and related ones, the reader is referred to Chapter 2 or Marais *et al.* (1983).

2. MODEL EVOLUTION

2.1 Early observations

Harold (1966), in an authoritative survey, reported that phosphorus accumulation in the form of polyphosphate (polyP) is widespread among micro-organisms, in bacteria, yeasts, fungi and photosynthetic algae. Harold, however, could not advance a substantive hypothesis as to what function was served by these accumulations. He noted that temporary limitations of the nutrients sulphur and nitrogen could result in accumulation of polyP in certain bacteria.

Levin and Shapiro (1965) demonstrated accumulation of phosphorus in a mixed liquor sample from an activated sludge system, under aerobic conditions. Further, they noted that in the mixed liquor samples phosphorus (P) is released to the bulk solution when the dissolved oxygen level falls. Shapiro (1967) showed that this release of P under anaerobic conditions could be reversed on subsequent aeration.

Fuhs and Chen (1975) conducted a wide-ranging investigation into the phenomena of P release and uptake. They observed the following:

- (1) Samples taken from a full-scale anaerobic/aerobic activated sludge plant (showing excess P removal) and from a laboratory scale aerobic plant fed on an artificial sewage (not showing excess P removal) were dosed with radioactively labelled glucose and aerated. The results indicated that the organism assembly receiving artificial substrate under completely aerobic conditions metabolized the glucose via the Entner-Duodoroff pathway, whereas the organism assembly from the anaerobic/aerobic plant indicated metabolism via the Embden-Meyerhof pathway. They then changed operation of the laboratory scale plant from aerobic to anaerobic/aerobic; after two weeks operation the system still showed no P release and uptake. They concluded that this system had a different species composition from the full-scale plant.
- (2) P removal appeared to be mediated by either a single microorganism or several closely related forms. From a series of isolation tests Fuhs and Chen concluded that *Acinetobacter* spp. probably was the principal organism mediating the P removal.
- (3) *Acinetobacter* spp. accumulated polyP and poly- β -hydroxybutyrate (PHB). Fuhs and Chen speculated that PHB storage could serve as an energy source for polyP formation.
- (4) In studying the nutritional requirements of an *Acinetobacter* strain isolated from a biological excess P removal activated sludge system they found this strain could not use glucose or similar compounds but grew on short chain fatty acids (SCFA). They concluded that anaerobic conditions preceding aerobiosis in sewage treatment could well be related to the appearance of *Acinetobacter* spp. - they saw the principal function of the anaerobic state as enabling a facultative anaerobic microflora to become established which produces short chain fatty acids to serve as substrate for *Acinetobacter* spp. Furthermore, from pure culture studies, they concluded that the anaerobic state takes no part in excess P removal *per se* and can be excluded provided SCFA are supplied to *Acinetobacter* spp. in the aerobic state. They found that if acetate was added to a pure culture of *Acinetobacter* spp. under aerobic conditions, P uptake took place.

Nicholls (1975) and Barnard (1976) from observations on P removal in full-scale plants concluded that, contrary to Fuhs and Chen, an anaerobic/aerobic sequence with substrate fed to the anaerobic zone were explicit requirements to obtain biological excess P removal. However, they could identify no correlation between the P release in the anaerobic reactor and P uptake and removal.

2.2 Model 1 (Nicholls and Osborn, 1979)

Nicholls and Osborn (1979) from a study of the literature and their own work noted a consistent association between polyP accumulation and organic carbon accumulation in the form of poly- β -hydroxybutyrate (PHB). They concluded that these high energy polymers were stored for survival purposes under the set of imposed anaerobic/aerobic conditions. They did not implicate any particular bacterial genus in polyP and PHB accumulation, but noted that in their systems *Acinetobacter*, a polyP and PHB accumulator, was abundantly present. They proposed a biochemical model in which they attempted to explain the functions of, and the connections between, PHB and polyP accumulations. They hypothesized the following model:

In the anaerobic phase:

- (1) Carbohydrate (glucose) is taken up by polyP organisms and metabolized, via the glycolytic (Embden-Meyerhof) pathway, to acetyl-CoA and electrons and protons.
- (2) The acetyl-CoA has two functions: First, to act as an electron and proton sink by its reduction to PHB. Second, to act as an energy source for ATP synthesis via deactivation to acetic acid.
- (3) The rôle of polyP is to serve as a source of P in the formation of ATP in (2) above.
- (4) The ATP generated in (2) above is utilized for cell maintenance via hydrolysis to ADP + Pi; such Pi is released to the medium.

In the aerobic phase:

- (1) The stored PHB is utilized as a carbon and energy source for cell function and an energy source for polyP generation and storage.

In support of their model Nicholls and Osborn set out quantitative biochemical pathways.

2.3 Model 2 (Rensink, 1981)

Rensink accepted the work of Fuhs and Chen (1975) that *Acinetobacter* spp. was the principal organism implicated in biological excess phosphorus removal and that these organisms only utilize short chain fatty acids (SCFA) as substrate. Also, he accepted Fuhs and Chen's hypothesis that the purpose of the anaerobic state was to enable an assemblage of facultative organisms to develop which produced SCFA to serve a substrate for the *Acinetobacter* spp. Furthermore he presupposed that *Acinetobacter* spp. is a slow growing obligate aerobe and in a purely aerobic environment would not be competitive for substrate with other obligate aerobes; however, by having the propensity for PHB storage in the anaerobic zone, competition for substrate in the aerobic zone is removed enabling the *Acinetobacter* spp. to survive in the anaerobic/aerobic cycle. With these ideas as background, taking due cognizance of the model of Nicholls and Osborn, he proposed the following conceptual model:

In the anaerobic phase:

- (1) Short chain fatty acids (for example acetate) serve as substrate for *Acinetobacter*.
- (2) The short chain fatty acids are stored as PHB, such a process requiring energy (ATP).
- (3) The energy (ATP) requirements in (2) above are supplied by breakdown (hydrolysis) of polyP to free phosphate, the phosphate being released to the bulk solution.

In the aerobic phase:

- (1) Rensink follows the proposals of Nicholls and Osborn.

Rensink did not propose any biochemical pathways for PHB synthesis from acetate or for polyP breakdown and P release.

The models of Rensink and Nicholls and Osborn differ in the following aspects:

- (1) SCFA as substrate for *Acinetobacter* (Rensink); - glucose as substrate for polyP organisms (Nicholls and Osborn).
- (2) Function of polyP is as - an energy source (Rensink); - a phosphorus source (Nicholls and Osborn).
- (3) Function of PHB is as - a carbon sink for *Acinetobacter* to survive in competition in the aerobic zone (Rensink); - a proton and electron sink for polyP organisms to survive an anaerobic stress (Nicholls and Osborn).

2.4 Model 3 (Marais, Loewenthal and Siebritz, 1983)

Marais *et al.* (1983) proposed that polyP accumulation in polyP organisms serves as an energy source for two purposes; maintenance during the anaerobic phase, and, PHB synthesis in the anaerobic phase thereby partitioning substrate in the anaerobic phase for the exclusive use of the polyP organisms subsequently in the aerobic phase, in this fashion gaining a positive advantage over non-polyP organisms in anaerobic/aerobic systems.

Marais *et al.* concurred with Rensink that polyP serves as an energy source for storage. They investigated theoretical biochemical pathways under anaerobic conditions, for PHB synthesis from the substrates glucose and acetate respectively. With *glucose* as substrate, accepting that the polyP organisms possess the glycolytic (Embden-Meyerhof) pathway, they proposed biochemical pathways whereby PHB could be synthesized. With *acetate* as substrate, however, they found it not possible to put forward pathways for PHB synthesis - conversion of acetate as far as acetoacetate only appeared to be feasible. The problem was that with acetate as substrate no source of protons and electrons, to reduce acetoacetate to PHB, was available. They were unable, therefore, to find an explanation for PHB storage under anaerobic conditions with acetate as substrate.

In some measure these speculations appeared to be supported by the work of Siebritz *et al.* (1983). They found that the mass of P removal in anaerobic/aerobic systems was proportional to an influent COD fraction called the 'readily biodegradable COD', a fraction that normally contains insignificant amounts of SCFA, (see Chapter 3). However an explanation was provided in Chapter 3. There it was shown that in the anaerobic phase with addition of acetate as substrate;

- (1) the mass of P release was proportional to the mass of acetate added, and
- (2) the rate of P release was zero order.

With addition of sewage as substrate it was shown that;

- (1) the P release, uptake and removal were proportional to the readily biodegradable COD concentration,
- (2) the rate of P release was first order with respect to the readily biodegradable COD concentration, and
- (3) the rate of P release was significantly lower than with acetate.

These observations suggested that the P release with sewage as influent was governed by the rate of SCFA generation by facultative organisms, as suggested by Fuhs and Chen (1975), from readily biodegradable substrate. The work of Meganck *et al.* (1985) and Brodisch (1985) supported these conclusions; they showed that anaerobic/aerobic systems developed organisms which converted sugars, and similar organic compounds, to SCFA in the anaerobic zone.

Juni (1978) reported that *Acinetobacter* spp. do not possess the glycolytic (Embden-Meyerhof) pathway. However some species (or strains) possess the Entner-Doudoroff pathway, a pathway which becomes inoperative when oxygen or nitrate is absent. Consequently these organisms cannot metabolize glucose under anaerobic conditions. Hence, the pathways proposed by Nicholls and Osborn (1979) and Marais *et al.* (1983) for PHB synthesis from glucose in the anaerobic zone, are not tenable. Evidence was presented in Chapter 4 that in completely aerobic systems receiving normal sewage, up to 60 percent of the organisms cultured aerobically from mixed liquor samples were *Acinetobacter* spp. This would indicate that there are strains or species of *Acinetobacter* which are *not* slow growing (as suggested by Rensink, 1981) and can compete successfully for substrate in purely aerobic systems. Furthermore these *Acinetobacter* must have been able to metabolize glucose or similar compounds aerobically via the Entner-Doudoroff pathway as the SCFA at best formed only a minor fraction of the biodegradable COD (see Chapter 3). However no polyP inclusions or enhanced P removal was observed in these purely aerobic systems. In Chapter 4 it was shown further that strains, isolated from systems exhibiting biological excess P removal and from

completely aerobic systems which did not show excess P removal, both had the propensity to accumulate polyP and PHB under standard culture conditions. It was concluded that the propensity to accumulate polyP and PHB is inherent in these strains and is induced when the appropriate anaerobic/aerobic conditions are imposed. The slow increase in P release, uptake and removal often observed when changing from completely aerobic to anaerobic/aerobic operation was attributed to the time factor necessary to develop relevant enzyme systems. These observations appear to negate Rensink's hypothesis that the *Acinetobacter* spp. are slow growing under aerobic conditions and that PHB accumulation in the anaerobic phase are essential for their survival under anaerobic/aerobic conditions.

2.5 Model 4 (Comeau, Hall, Hancock and Oldham, 1985)

In 1985 Comeau *et al.* proposed a conceptual model that resolved some of the difficulties and inconsistencies raised above. Comeau *et al.* (1985), following Rensink, accepted acetate as substrate and that polyP serves as an energy source for activation of acetate to acetyl-CoA in the anaerobic state. To overcome the problem that troubled Marais *et al.*, namely that in the anaerobic state with acetate as substrate no source of electrons and protons was available to reduce acetyl-CoA to PHB, they proposed that these can be supplied from the tricarboxylic acid (TCA) cycle. However they did not elaborate on the biochemical pathways by means of which this can be achieved. They noted further that acetate is taken up by the organism in the undissociated acetic acid (HAc) form, as generally accepted in biochemistry. This causes the proton motive force (pmf) across the cytoplasmic membrane, essential to cell function (for function see Comeau *et al.*, 1985), to be dissipated. To re-establish the pmf they proposed that the polyP serves as the energy source for translocation of protons. Comeau *et al.* showed further that for each P released the cations Mg^{2+} , K^{+} and Ca^{2+} are released in the molar ratios (cation:phosphate) 0,27; 0,28 and 0,02 respectively. In terms of charge, one positive cation charge is released with every phosphate.

They described the biochemical pathways only in outline.

Qualitatively the model of Comeau *et al.* provided a framework for explaining anaerobic/aerobic behaviour of excess P release and uptake. However, the model was incomplete in that it did not provide quantitative information on the pathways and control mechanisms governing the biochemical reactions under different sets of imposed conditions of substrate and oxygen tension. Furthermore, an analysis of the mechanism proposed for maintaining the pmf in the anaerobic zone gave rise to

charge and proton imbalances across the cytoplasmic membrane.

In the rest of this chapter we will be concerned with modifying and extending the model of Comeau *et al.*, to overcome the objections raised above. In this endeavour the genus *Acinetobacter* will be accepted as a typical polyP organism. Although Brodisch and Joyner (1983) have shown that a number of genera are associated with biological excess phosphorus removal the genus *Acinetobacter* has been identified to be principally responsible for P release and P removal in appropriately designed activated sludge systems, Buchan (1983).

3. PRELIMINARY CONSIDERATIONS

3.1 Characteristics of *Acinetobacter* spp.

In Chapter 4 some characteristics of *Acinetobacter* spp. have been listed. For convenience these are repeated here together with others relevant to the purpose of this chapter.

Acinetobacter spp. are ubiquitous in nature and can be readily isolated from soil, water and sewage (Warskow and Juni, 1972; Juni, 1978). All *Acinetobacter* spp. are gram negative, non-motile, catalase positive and oxidase negative (Henriksen, 1973; Juni, 1978; Fricke *et al.*, 1982). The genus requires oxygen for catabolic metabolism, Juni (1978); there are however some species within the genus which can utilize nitrate as an electron acceptor where oxygen is not present (Lötter, 1985). A characteristic of the genus is that they utilize sugars exclusively via the Entner-Doudoroff pathway - a pathway that is inoperative under anaerobic conditions. As a consequence they cannot produce energy from fermentation due to the absence of the glycolytic or similar metabolic pathways - they are able to utilize sugars only under aerobic (and anoxic) conditions. Furthermore, the genus is capable of storing phosphorus as polyP (Deinema *et al.*, 1980; Buchan, 1983) and organic carbon as poly- β -hydroxybutyrate (Lawson and Tonhazy, 1980). It is this propensity to store P and organic carbon which, with the appropriate process configuration, is utilized to effect excess P removal. Each of these metabolic processes and their regulation is discussed below.

3.2 Carbon metabolism

Carbon metabolism of *Acinetobacter* spp. in activated sludge incorporates three main metabolic pathways, namely; (1) the tricarboxylic acid cycle, (2) glyoxylate cycle and (3) poly- β -hydroxybutyrate synthesis and degradation. The pathways and the relevant controls are discussed below.

- (1) Tricarboxylic Acid (TCA) cycle: The main steps of the TCA cycle are shown in Fig 5.1. The figure illustrates that, for an input of one molecule of acetyl-CoA, in a complete cycle of the pathway, two molecules of carbon dioxide are lost and eight electrons (and protons) are captured by reduction of three molecules of nicotinamide adenine dinucleotide (NAD) to the reduced form (NADH) and one molecule flavin adenine dinucleotide (FAD) to the reduced form (FADH). Under aerobic conditions these reduced coenzymes donate the electrons and protons to oxygen and adenosine triphosphate (ATP) is generated from this redox reaction. Organic intermediates are formed at different stages of the TCA cycle; these become available to the organism if required in which event the full cycle is not completed. Thus the function of the TCA cycle under aerobic conditions is for energy generation as well as supplying carbon intermediates for anabolism. Under anaerobic conditions no terminal electron acceptor is available and, unless some alternative electron sink is found, then the NADH/NAD (or NADPH/NADP) ratio increases, and ATP/ADP ratio decreases. The TCA cycle is regulated by these ratios; high values for these ratios have an inhibitory effect on the cycle; this inhibition has been observed in *Acinetobacter* spp. High values of NADH/NAD inhibit the enzymes citrate synthase (Weitzman and Jones, 1968; Weitzman and Dunmore, 1969) and α -ketoglutarate dehydrogenase (Weitzman, 1972); high values of ATP/ADP inhibit the enzymes isocitrate dehydrogenase (Parker and Weitzman, 1970) and citrate synthase (Weitzman and Dunmore, 1969); two isoenzymes of isocitrate dehydrogenase have been identified in *Acinetobacter* spp. and are termed isoenzyme I and isoenzyme II - isoenzyme I is regulated by NADPH/NADP (Self and Weitzman, 1972) and isoenzyme II by NADH/NAD (Weitzman, 1972).
- (2) Glyoxylate cycle: This cycle is shown in Fig 5.1. The principal function of this cycle is anaplerotic, that is, to supply intermediates to the TCA cycle from two carbon (C2) units. The two key enzymes in this cycle, isocitrate lyase and malate synthase, have been detected in *Acinetobacter* spp. (Sturm *et al.*, 1970). In most organisms regulation of the cycle is effected by three carbon (C3) compounds. In *Acinetobacter* spp. it is the four carbon (C4) intermediates of the TCA cycle which regulate activity of the enzyme isocitrate lyase (Bell and Herman, 1967; Herman and Bell, 1970) and thus regulate the glyoxylate cycle. As the TCA cycle is controlled by the NADH/NAD and ATP/ADP ratios, and as the TCA cycle intermediates

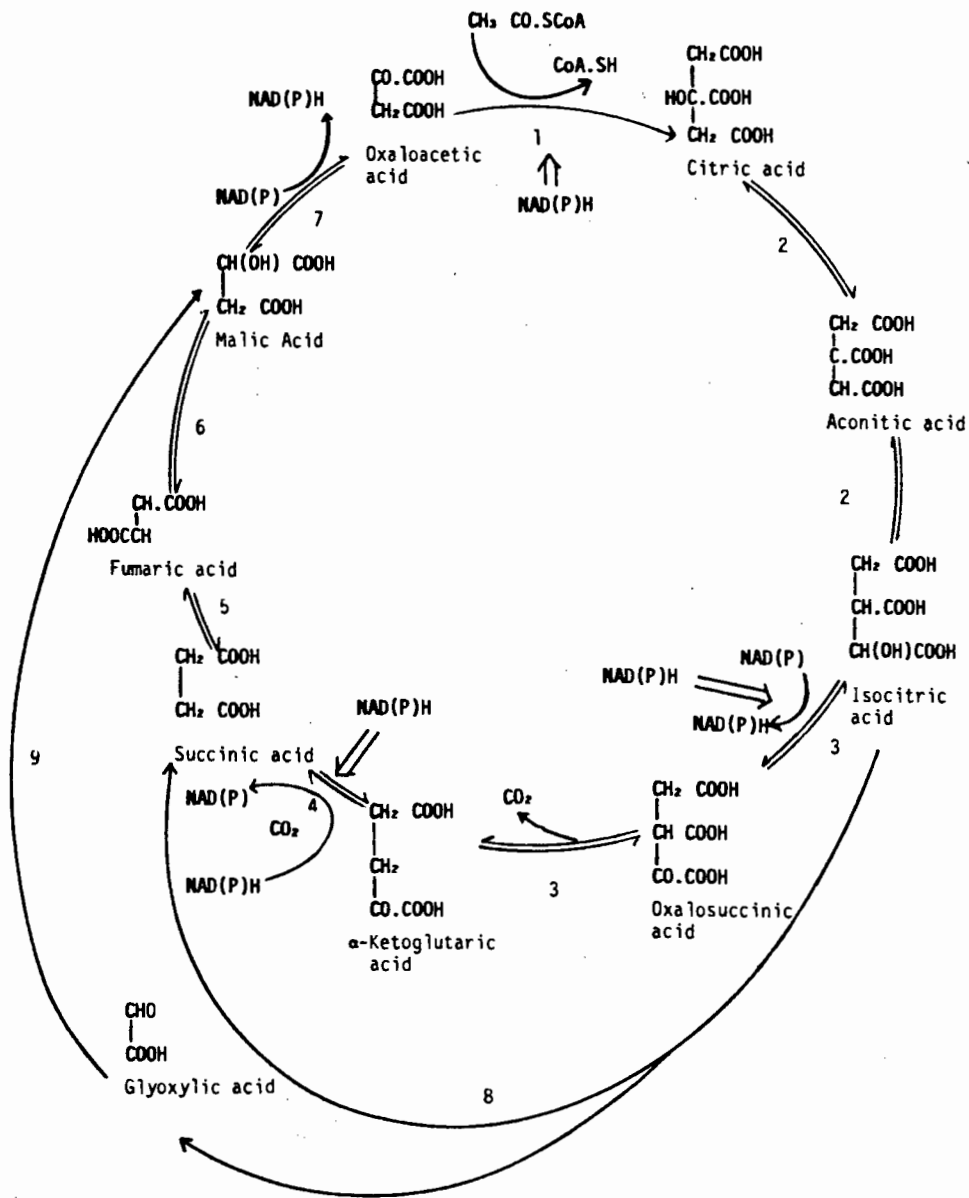


Fig 5.1: Tricarboxylic acid and glyoxylic acid cycles

Key to enzymes:

1. Citrate synthase
2. Aconitase
3. Isocitrate dehydrogenase
4. α-ketoglutarate dehydrogenase
5. Succinate dehydrogenase
6. Fumarase
7. Malate dehydrogenase
8. Isocitrate lyase
9. Malate synthase

⇒ Indicates inhibition

regulate the glyoxylate cycle, this cycle is indirectly controlled by the NADH/NAD and ATP/ADP ratios.

- (3) Poly- β -hydroxybutyrate (PHB) synthesis and degradation: Synthesis and degradation pathways of PHB are shown in Fig 5.2. Regulation of these pathways has not been studied specifically in *Acinetobacter* spp. However, studies on these pathways in numerous other bacterial species invariably show the same system of regulation (Dawes and Senior, 1973); we therefore assume that this holds also for *Acinetobacter* spp. The synthesis and degradation of PHB proceeds via separate metabolic pathways, as follows:

- (i) **Synthesis of PHB:** Referring to Fig 5.2, synthesis of PHB takes place via activation of acetate to acetyl-CoA (mediated by the enzyme thiokinase), condensation of two molecules of acetyl-CoA to form acetoacetyl-CoA (catalysed by the enzyme ketothiolase) and reduction of acetoacetyl-CoA to hydroxybutyryl-CoA (catalysed by the enzyme β -hydroxybutyrate dehydrogenase) which is then polymerized to form PHB (catalysed by the enzyme hydroxybutyryl-CoA polymerase). The synthesis of PHB acts as a sink for protons and electrons and organic carbon. The synthesis pathway is stimulated by a high NADH/NAD ratio (or NADPH/NADP) and also by high concentrations of acetyl-CoA (Dawes and Senior, 1973). An inhibitory effect on the synthesis pathway has been observed, that of a high concentration of CoASH via its action on the enzyme ketothiolase (Dawes and Senior, 1973). One therefore expects this synthesis pathway to be functional where a high concentration of organic carbon is available to the organism, and no external electron sink (i.e. an anaerobic state) is present.
- (ii) **Degradation of PHB:** Referring to Fig 5.2, the degradation pathway proceeds via the hydrolysis of PHB to free β -hydroxybutyrate (catalysed by the enzyme PHB depolymerase), oxidation of this acid to acetoacetate (catalysed by NAD β -hydroxybutyrate dehydrogenase) and activation of acetoacetate to acetoacetyl-CoA (catalysed by acetoacetate succinyl-CoA CoA transferase) which, with activated cleavage, forms two molecules of acetyl-CoA (catalysed by ketothiolase). These end products now may enter the TCA cycle. Regulation of this degradation pathway is via inhibition

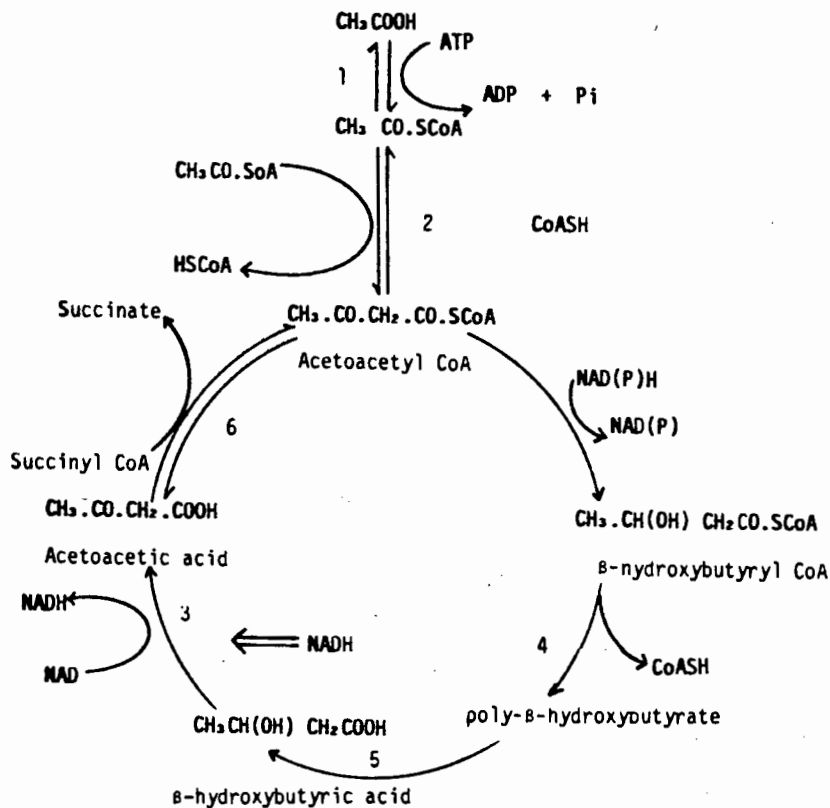


Fig 5.2: Synthesis and degradation of poly- β -hydroxybutyrate

Key to enzymes:

1. Thiokinase
2. β -ketothiolase
3. β -hydroxybutyrate dehydrogenase
4. β -hydroxybutyryl CoA polymerase
5. Poly- β -hydroxybutyrate depolymerase
6. Acetoacetate succinyl CoA:CoA transferase

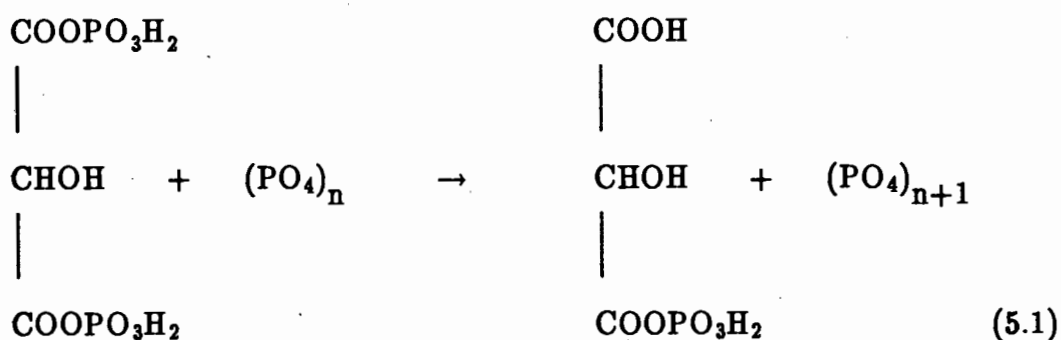
\Rightarrow Indicates inhibition

of NAD β -hydroxybutyrate dehydrogenase (which catalyses the oxidation of the free acid to acetoacetate) by high concentrations of pyruvate and/or a high NADH/NAD ratio (Dawes and Senior, 1973). One therefore expects this PHB degradation pathway to be functional under conditions where extracellular organic substrate concentration is low and a terminal electron acceptor (i.e. an aerobic state) is present.

3.3 Phosphorus metabolism

Phosphorus is accumulated by *Acinetobacter* spp. as polyphosphate and stored in intracellular metachromic granules which may occupy up to 60 percent of the total microorganism volume (Buchan, 1981). Polyphosphate contains a large negative charge which probably is stabilized by the cations Mg^{2+} , K^+ and Ca^{2+} . Polyphosphate (polyP) metabolism within *Acinetobacter* spp. occurs via two pathways; (1) a synthesis pathway and (2) a degradation pathway.

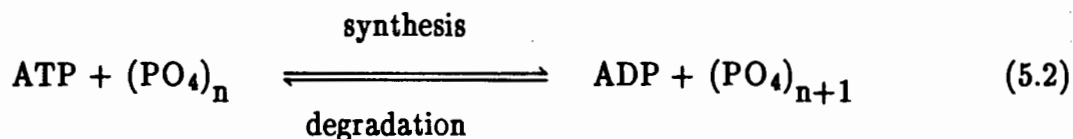
- (1) Synthesis of polyP: Two pathways of polyP synthesis have been observed in microorganisms, neither of which have been studied specifically in *Acinetobacter* spp. The first pathway is via phosphorylation of the polyphosphate by 1,3 diphosphoglycerate (catalysed by the enzyme diphosphoglycerate: polyphosphate transferase) - a pathway not widely observed. The reaction is as follows:



The enzyme catalysing this reaction has been observed only in a narrow spectrum of micro-organisms including *Escherichia coli* (Nesmeyanova *et al.*, 1974), *Micrococcus lysodeikticus*, *Propionibacterium shermanii* and *Neurospora crassa* (Kulaev and Bobyk, 1971; Kulaev, 1973).

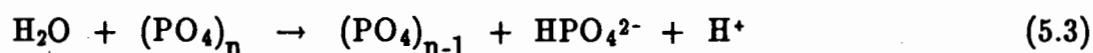
The second polyP synthesis pathway is via phosphorylation of the polyP by ATP (catalysed by the enzyme ATP:polyphosphate phosphotransferase).

The reaction is as follows:



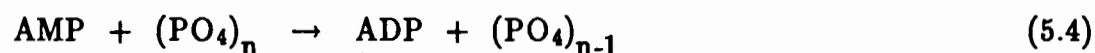
This pathway has been identified in a wide variety of organisms (Harold, 1966; Suzuki *et al.*, 1972; Felter and Stahl, 1970; Kulaev, 1975) and is the principal mechanism of polyP synthesis. Regulation of this pathway is via the ATP/ADP ratio; polyP synthesis is inhibited by a low ATP/ADP ratio (Kornberg, 1957; Zaitseva and Belozerskii, 1960; Felter and Stahl, 1970), a condition likely to be encountered where ATP is low, i.e. under anaerobic conditions where no external electron acceptor is present, irrespective of the substrate concentration surrounding the organism.

- (2) Degradation of polyP: The degradation of polyP to free phosphate radicals has been observed to take place via three reaction pathways in micro-organisms. First, the reverse reaction of that set out in the reaction Eq (5.2), also catalysed by the enzyme ATP:polyphosphate phosphotransferase, (Kornberg, 1957; Harold and Harold, 1965; Kulaev, 1975; Shabalin *et al.*, 1977). This degradation pathway is inhibited by a high ATP/ADP ratio (Harold and Harold, 1965), a situation likely to arise when ATP level is high, that is, under aerobic conditions with internal stored PHB or external substrate (e.g. acetate). Second, the hydrolysis of polyP to release the free phosphate radical (catalysed by the enzyme polyphosphatase; Harold, 1964; Severin *et al.*, 1976) as follows:



Regulation of this pathway is effected by the cellular phosphate concentration (Felter and Stahl, 1970; Nesmeyanova *et al.*, 1974). A high cellular phosphate concentration will inhibit this reaction.

Third, phosphorylation of AMP by polyP (catalysed by the enzyme polyphosphate:AMP phosphotransferase) as follows:



The reaction pathway has been observed in mycobacteria (Harold, 1966; Kulaev, 1975). The regulation of the reaction pathway has not been studied.

Having established the mechanisms of carbon and phosphorus metabolism it is possible now to interlink these pathways under the conditions imposed by the activated sludge system to describe *Acinetobacter* spp. behaviour.

4. BIOCHEMICAL MODEL

The development of the model, set down below, is formulated for the environmental conditions imposed on *Acinetobacter* spp. that result in biological excess P removal, i.e. an anaerobic/aerobic sequence. Fundamental in the model development is the effect of the anaerobic and aerobic phases (in the anaerobic/aerobic sequence) on the intracellular ratios NADH/NAD and ATP/ADP, and the influence of these ratios in the biochemical regulation of carbon and phosphorus metabolic pathways.

4.1 Anaerobic phase

In this phase no external electron acceptor is available (neither oxidized nitrogen species nor oxygen), further, the organisms are in an environment containing a relatively high concentration of readily assimilable organic substrate.

Consequences of no external electron acceptor are that the NADH/NAD ratio increases, and no oxidative phosphorylation (ATP generation) takes place. The lack of oxidative phosphorylation causes the ATP/ADP ratio to decrease. The effects of these changes in the ratios of NADH/NAD and ATP/ADP are to inhibit and stimulate the TCA cycle respectively (cf. regulation of TCA cycle above). Clearly if an electron sink is available to the NADH (causing a decrease in the NADH/NAD ratio) inhibition of the TCA cycle is removed and further NADH generation by the TCA cycle is possible. Decrease in the ATP/ADP ratio stimulates ATP production via hydrolysis of stored polyP and transfer of the high energy phosphoryl group to ADP.

Consequence of the high organic carbon concentration is that provided both an electron and energy source are available, i.e. provided the NADH/NAD and ATP/ADP ratios are sufficiently high, the organism can take up the carbon and store it as PHB. The source of electrons will vary depending on substrate. The most widely observed substrate to *Acinetobacter* spp. is acetate, consequently the metabolic fate of this substrate is considered first:

- (1) A high external concentration of acetate allows passive diffusion of acetate into the cell, i.e. without expenditure of energy (Konings *et al.*, 1981).
- (2) Activation of acetate to acetyl-CoA, by coupled ATP hydrolysis, decreases the ATP/ADP level to such a degree that ATP formation is stimulated via polyP degradation and the transfer of the phosphate and energy to ADP, see Eq 5.2 (thermodynamic control of this degradation process, and its reversal, are considered in Appendix C). The hydrolysis of polyP to form ATP and the subsequent utilization of ATP increases the intracellular concentration of free phosphate. With polyP degradation the cations stabilizing the negative charge on the polyP chain also are released increasing the intracellular cation concentration. The phosphate and cations (M^+) are subsequently released to the bulk solution. The biochemical interaction between the concomitant acetate uptake and phosphate and cation release is described after (4) below.
- (3) The high concentration of acetyl-CoA and a concomitant high NADH/NAD ratio stimulates PHB synthesis. Referring to Fig 5.2 it is evident in the synthesis of PHB that NADH is oxidized to NAD, with the electrons (and protons) reducing acetoacetyl-CoA to β -hydroxybutyryl-CoA. PHB thus acts as an electron sink and this decreases the NADH/NAD ratio, removing the inhibition of the TCA cycle (and associated glyoxylate cycle) for further electron generation which in turn increases the NADH/NAD ratio. This interaction between the TCA cycle and PHB synthesis, mediated by the NADH/NAD ratio, ensures electron and proton generation to store all acetate taken up as PHB. Summarizing, for acetate uptake under anaerobic conditions a fraction of the acetate is reduced to PHB, and a fraction is oxidized in the TCA cycle to supply the electrons and protons for the reductive process via NADH formation. Stoichiometrically for acetate as carbon source about 89 percent of the carbon is stored as PHB and 11 percent reappears as CO_2 ; however, *all* the electrons and protons of the acetate taken up reappear in PHB. A diagram of the stoichiometry is set out in Fig 5.3.
- (4) Formation of PHB lowers the intracellular concentration of acetate, allowing continued diffusion of acetate into the cell.

The mechanisms outlined above are in agreement with observed enzyme control systems as set out earlier in this chapter. However, the mechanisms do not explain

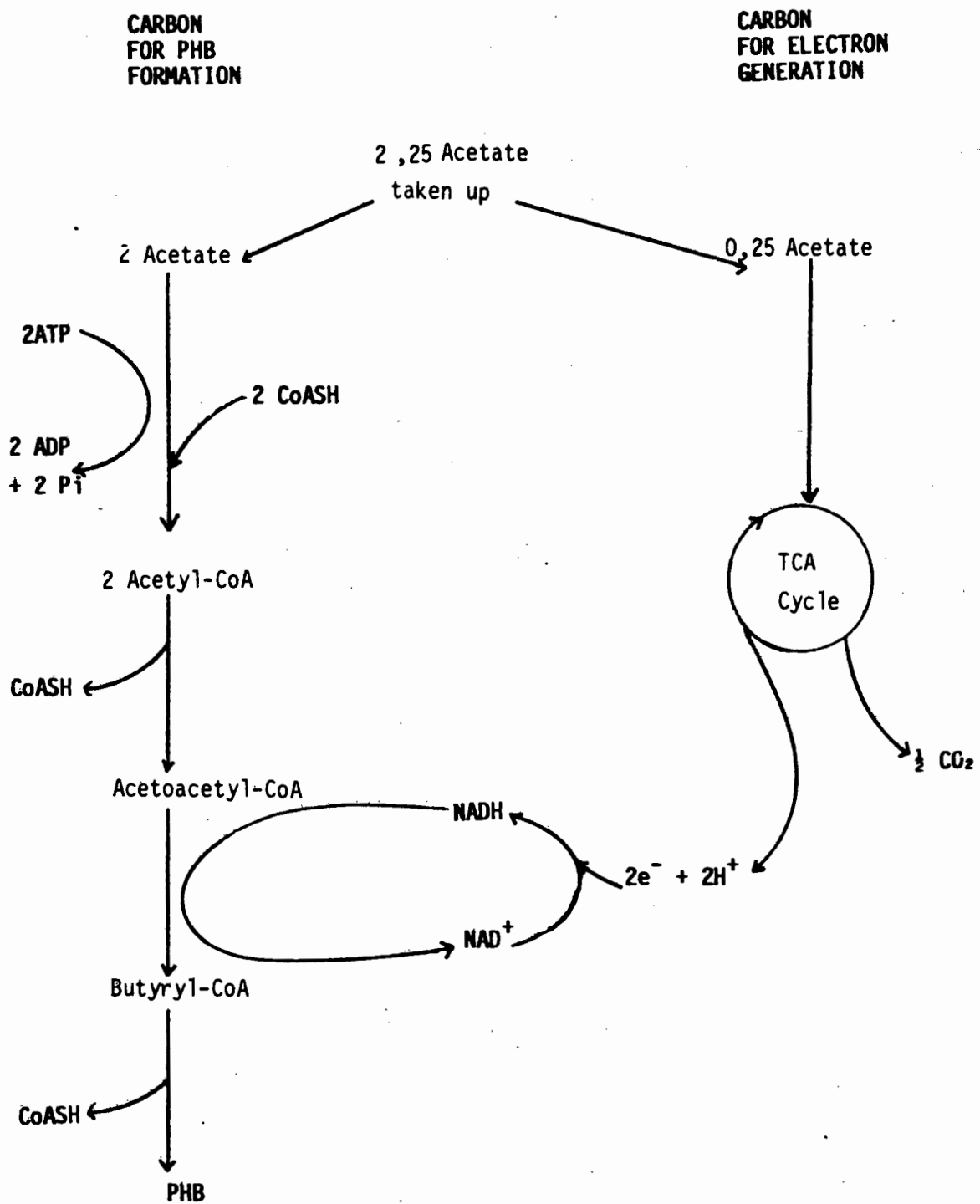


Fig 5.3: Stoichiometry of acetate utilization in PHB formation under anaerobic conditions where acetate is the substrate.

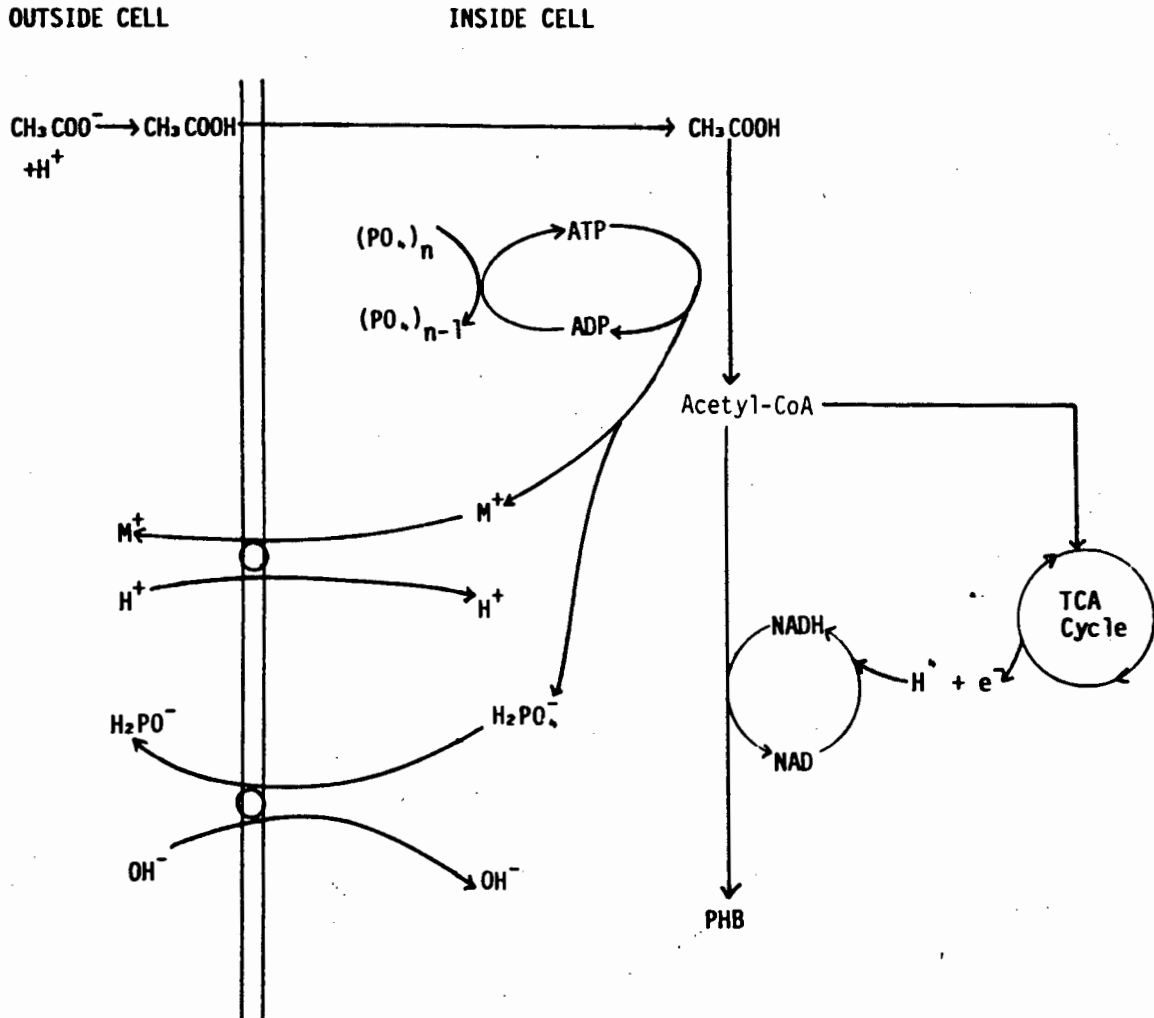
the biochemical links between acetate uptake and phosphate and cation release. Two fundamental biochemical principles link the release of phosphate and cations to the uptake of acetate across the cytoplasmic membrane. First, the generation of intracellular phosphate and cations from polyP hydrolysis needed to provide energy for acetate storage [see (2) above]; second, uptake of acetate in the acetic acid form dissipates the proton motive force (pmf), the force being vital as a potential energy source for translocation of molecules across the cytoplasmic membrane of the cell. This pmf ($\Delta\mu_H$) has both a pH and a charge ($\Delta\psi$) component according to Eq (5.5), Padan *et al.* (1976),

$$\Delta\mu_H = \Delta\psi + \frac{2,3RT}{F} \Delta\text{pH} \quad (5.5)$$

For continued acetate uptake *inter alia*, the dissipated pmf must be restored (Comeau *et al.*, 1985). It is proposed that this is achieved by the concomitant release of phosphate and cation, and uptake of hydroxyl ions and protons from the bulk solution, as set out in Fig 5.4. Referring to this figure,

- (1) phosphate release occurs via hydroxyl mediated antiport protein carrier, a transport system commonly observed in microorganisms (Harold and Spitz, 1975),
- (2) the cation (M^+) release occurs via a proton mediated antiport protein carrier, and
- (3) acetic acid uptake is by passive diffusion.

If the pmf is to be maintained, then, from Eq (5.5), the pH and charge difference between inside and outside the cell must be maintained. The question is whether the proposed mechanisms set out in Fig 5.4 satisfy these requirements. In terms of the mechanisms in Fig 5.4 the extracellular changes in alkalinity, acidity and charge are set down in Table 5.1 and the intracellular changes in Table 5.2. These tables show that charge neutrality is maintained both extracellularly and intracellularly. With regard to alkalinity and acidity, intracellularly there is no change; extracellularly the alkalinity and acidity each increase by one mole for every mole of acetic acid uptake. In the pH region 6.8–7.2 the buffering capacity of the phosphate system dominates (pK value of about 7). Consequently in this pH region for equal increases in alkalinity and acidity, one can show that the pH change will be zero or insignificant

**Fig 5.4:**

Translocation of acetate, phosphate and cations across the cytoplasmic membrane under anaerobic conditions. For completeness the polyphosphate degradation and polyhydroxybutyrate synthesis to attain this translocation are shown in outline.

Table 5.1: Extracellular (bulk solution) alkalinity, acidity and charge changes for the proposed model under anaerobic conditions with uptake of one mole of acetic acid.

Process	Alk (mol)	Nett change in Acid (mol)	Charge (mol)
HAc moves into cell and condensed into PHB	0	-1	0
H ₂ PO ₄ ⁻ moves out of cell	+1	+2	-1
OH ⁻ moves into cell	-1	+1	+1
M ⁺ moves out of cell	0	0	+1
H ⁺ moves into cell	+1	-1	-1
Sum of changes	+1	+1	0

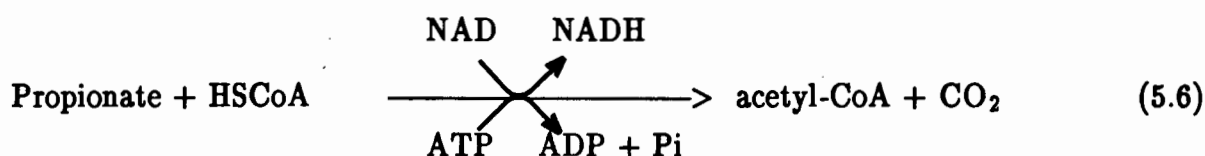
Table 5.2: Intracellular alkalinity, acidity and charge changes for the proposed model under anaerobic conditions with uptake of one mole of acetic acid.

Process	Alk (mol)	Nett change in Acid (mol)	Charge (mol)
HAc into cell	0	-1	0
HAc into PHB synthesis	0	-1	0
*PolyP hydrolysis: (PO ₄) _n → (PO ₄) _{n-1} + H ₂ PO ₄ ⁻ + M ⁺	+1	+2	0
H ₂ PO ₄ ⁻ out of cell	-1	-2	+1
OH ⁻ into cell	+1	-1	-1
M ⁺ out of cell	0	0	-1
H ⁺ into cell	-1	+1	+1
Sum of changes	0	-0	0

*PolyP hydrolysis to free phosphate involves ATP formation and subsequent utilization as intermediate steps.

(Wentzel *et al.*, 1988a). This is confirmed by experimental observation, see Chapter 6.

The anaerobic biochemical model above was developed for acetate as substrate. For other observed organic substrate sources, e.g. propionate, butyrate, etc. the mechanisms are similar. However, for these the generation of NADH may not require the TCA cycle. For example, for propionate the observed generation of NADH is as follows (Hodgson and McGarry, 1968)



The generation of NADH maintains the NADH/NAD ratio at a level which inhibits the TCA cycle, and stimulates PHB synthesis (cf. section on carbon metabolism above). ATP requirements are supplied by polyP cleavage, as in the case of acetate.

4.2 Aerobic phase

In this phase an external electron acceptor is available in the form of molecularly dissolved oxygen; furthermore the organisms are in an environment where organic substrate in the bulk liquid surrounding them is limited (readily available (soluble) COD having been assimilated in the preceding anaerobic phase). However the organisms do possess internally stored PHB. A consequence of the presence of an external electron acceptor is a reduction in the NADH/NAD ratio, and, with concomitant oxidative phosphorylation (ATP generation), the ATP/ADP ratio increases. The decrease in the NADH/NAD ratio stimulates the degradation of PHB, the TCA cycle and associated glyoxylate pathway, (cf. aerobic section on carbon metabolism above). Degradation of PHB to acetate provides a carbon and energy source for cell function.

With regard to polyP degradation/synthesis, the high ATP/ADP ratio stimulates polyP synthesis, as shown in Eq 5.2. A further important result of a high ATP/ADP ratio is that it enables the organism to establish any required pmf and to utilize ATP for molecule translocation, i.e. the difference between extracellular and intracellular pH no longer is the determining factor for transport function whereas in the anaerobic state it is (see above).

The proposed mechanisms of phosphate and cation uptake for polyP synthesis are

given in Fig 5.5. Phosphate uptake occurs via the hydroxyl mediated antiport and cation uptake via the proton mediated antiport. Intracellular and extracellular changes in alkalinity, acidity and charge for the proposed mechanisms are given in Tables 5.3 and 5.4 respectively. Charge neutrality is maintained both extracellularly (Table 5.3) and intracellularly (Table 5.4). With regard to alkalinity and acidity; intracellularly there is no change; extracellularly the alkalinity and acidity decrease by one mole and two moles respectively for each mole of phosphate taken up. As for the anaerobic zone, pH buffering by the phosphate system ($pK \sim 7$) dominates. It can be shown that for a decrease in alkalinity by one mole, and a decrease in acidity by two moles, the pH of the mixed liquor from the anaerobic zone will increase in the aerobic zone if the anaerobic pH > 6.8 (Wentzel *et al.*, 1988a). Such pH behaviour conforms with observations in aerobic zones of *Acinetobacter* spp. enhanced culture activated sludge systems, see Chapter 6.

5. IMPLICATIONS

5.1 Anaerobic/aerobic systems

In terms of the model set out above, the behaviour of *Acinetobacter* spp. in sequential anaerobic/aerobic activated sludge systems can be predicted and evaluated against observations in the literature on these systems.

In the anaerobic zone:

- (1) Decrease in SCFA in the bulk solution (Rensink, 1981).
- (2) Increase in phosphate concentration in the bulk solution (extensively reported).
- (3) Increase in cation concentration in the bulk solution, in particular Mg^{2+} and K^+ (Comeau *et al.*, 1985).
- (4) Molar ratio of SCFA decrease to phosphate increase in the bulk solution approximately 1:1 (Fukase *et al.*, 1982; Wentzel *et al.*, 1985).
- (5) Charge ratio of cation increase to phosphate increase in the bulk solution is one mole positive charge increase per mole phosphate increase (Comeau *et al.*, 1985).

Outside Cell

Inside Cell

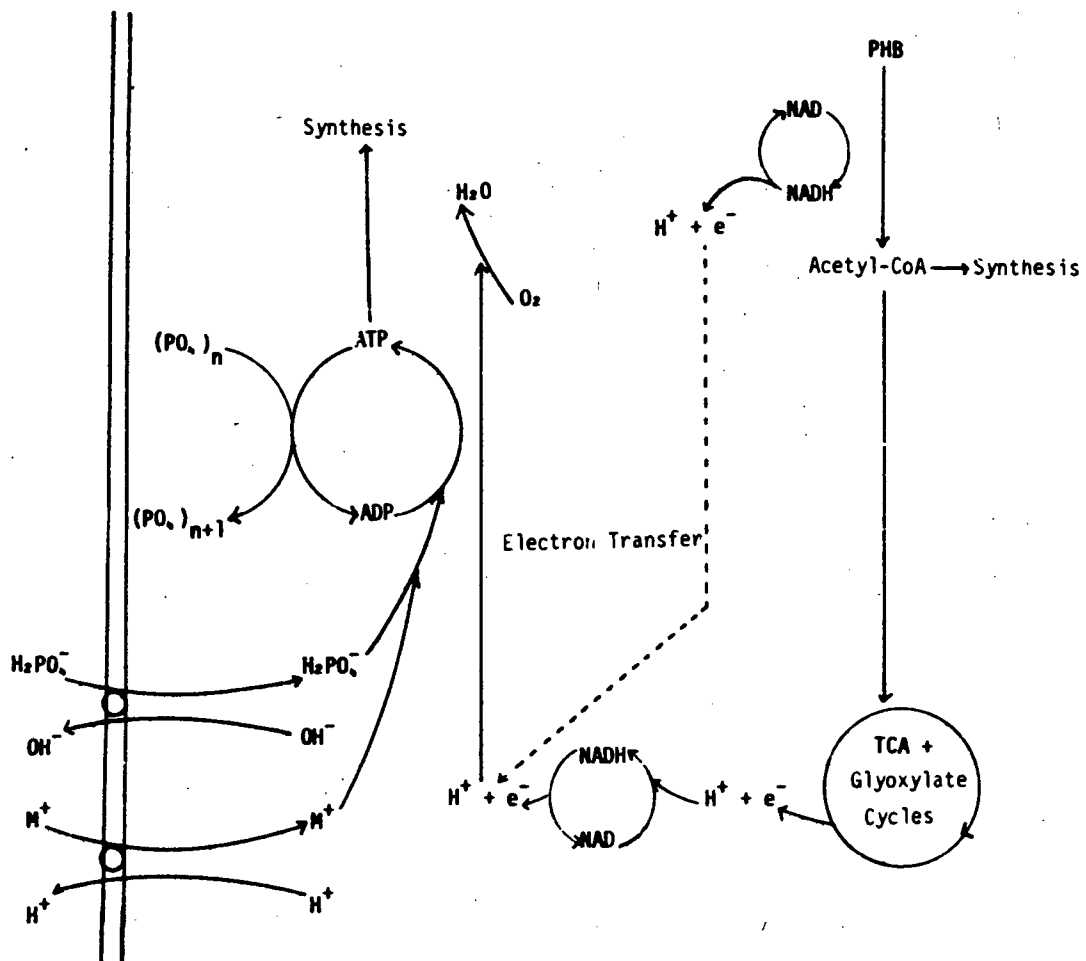


Fig 5.5: Translocation of phosphate and cations across the cytoplasmic membrane under aerobic conditions. For completeness the polyphosphate synthesis and polyhydroxybutyrate degradation to attain this translocation are shown in outline.

Table 5.3: Extracellular (bulk solution) alkalinity, acidity and charge changes for the proposed model under aerobic conditions using PHB as substrate.

Process	Alk (mol)	Nett change in Acid (mol)	Charge (mol)
H_2PO_4^- out of cell	-1	-2	+1
OH^- into cell	+1	-1	-1
M^+ into cell	0	0	-1
H^+ out of cell	-1	+1	+1
Sum of changes	-1	-2	0

Table 5.4: Intracellular alkalinity, acidity and charge changes for the proposed model under aerobic conditions using PHB as substrate.

Process	Alk (mol)	Nett change in Acid (mol)	Charge (mol)
H_2PO_4^- into cell	+1	+2	-1
OH^- out of cell	-1	+1	+1
M^+ into cell	0	0	+1
H^+ out of cell	+1	-1	-1
*PolyP synthesis: $(\text{PO}_4)_n + \text{H}_2\text{PO}_4^- \text{M}^+ \rightarrow (\text{PO}_4)_{n+1}$	-1	-2	0
Sum of changes	0	-0	0

*PolyP synthesis involves the intermediate step of ATP formation by oxidative phosphorylation, and the transfer of the high energy phosphoryl group to the polyP chain.

- (6) Increase (synthesis) of intracellular PHB (Fukase *et al.*, 1982; Hart and Melmed, 1982).
- (7) Decrease (degradation) of intracellular polyP (Murphy and Lötter, 1986).
- (8) No net change in bulk solution pH (Wentzel *et al.*, 1988b).

In the aerobic zone:

- (1) Decrease in phosphate concentration in bulk solution (extensively reported).
- (2) Decrease in cation concentration in the bulk solution, in particular Mg^{2+} and K^+ (Comeau *et al.*, 1985).
- (3) Charge ratio of cation decrease to phosphate decrease in the bulk solution is one mole positive charge decrease per mole phosphate decrease (Comeau *et al.*, 1985).
- (4) Decrease (degradation) in intracellular PHB (Hart and Mehmed, 1982).
- (5) Increase (synthesis) in intracellular polyP (Murphy and Lötter, 1986).
- (6) Increase in bulk solution pH (Wentzel *et al.*, 1988b).

It is evident that the behavioural patterns predicted by the model in the anaerobic/aerobic sequence conform with observations.

The model can also be applied to describe *Acinetobacter* spp. behaviour in systems and environments that do not include anaerobic/aerobic sequences.

5.2 Completely aerobic environment

Pure cultures of *Acinetobacter* spp. developed aerobically have been shown to accumulate polyP and PHB (for example, see Chapter 4). In the culture conditions, *Acinetobacter* spp. invariably are placed in an environment where both a suitable external carbon source (e.g. acetate) in high concentration and an electron acceptor (oxygen) are present. In terms of the model these conditions should stimulate polyP and PHB accumulation. The presence of an external electron acceptor momentarily decreases the NADH/NAD ratio. The initial decrease in NADH/NAD ratio and the

presence of a high substrate concentration both stimulate the TCA cycle. The substrate taken up is utilized for two purposes;

- (1) as a carbon source for anabolism, and
- (2) metabolism by the TCA cycle for energy production and production of specific carbon compounds.

The high concentration of acetate entering the TCA cycle increases the ATP/ADP ratio to a level sufficiently high to stimulate polyP synthesis and also increases the NADH/NAD ratio. Thus, if the concentration of acetate and thus acetyl-CoA is high, PHB synthesis will be stimulated, with TCA cycle operation supplying protons and electrons for the reduction of acetate to PHB.

Let us now consider what happens in a completely aerobic system with, say, a sludge age of 10–20 days. In the mixed liquor the substrate concentration is low due to competition between organisms for the substrate. In such systems it was shown in Chapter 4 that *Acinetobacter* spp. are present in appreciable concentrations and it was concluded that the *Acinetobacter* spp. are competitive for substrate (via the Entner-Doudoroff pathway) with other aerobes, even though the substrate concentration is low. However no polyP or PHB accumulation has been observed. This behaviour can be explained in terms of the model as follows: The low substrate concentration causes that the ATP/ADP ratio is not elevated sufficiently for polyP synthesis, and NADH/NAD ratio is kept low; low substrate concentration and low NADH/NAD both inhibit PHB synthesis.

If the *Acinetobacter* spp. from the aerobic long sludge age system above are isolated and cultured aerobically they accumulate polyP and PHB, see Chapter 4. Clearly the propensity to accumulate polyP and PHB is an inherent characteristic of the organism; it requires only a stimulus to trigger the biochemical pathways necessary for polyP and PHB accumulation. This stimulus, we have seen is a high ATP/ADP ratio (for polyP accumulation) and a high NADH/NAD ratio plus a high substrate concentration (for PHB accumulation). If the extracellular substrate concentration is high, as in pure cultures, or the intracellular substrate concentration (i.e. PHB) is high, as in the aerobic zone of anaerobic/aerobic systems, then the ATP/ADP ratio is elevated sufficiently for polyP synthesis. If the extracellular substrate concentration is high and an external electron acceptor is present, then both PHB and polyP are accumulated.

5.3 Endogenous mass loss

In order to investigate the endogenous mass loss phenomenon in *Acinetobacter* spp. a high concentration of this organism was developed in a UCT (or modified Bardenpho) system by addition of acetate as substrate to the anaerobic reactor of an anaerobic/aerobic sequence, see Chapter 6. An analysis of the population structure indicated that more than 90 percent of the organisms cultured aerobically from system samples were *Acinetobacter* spp. When a sample from the aerobic zone of the system was aerated under batch conditions and the phosphate (P) concentration of the bulk solution and oxygen utilization rates (OUR) were monitored with time, the response showed a two phase behaviour: Initially there was a rapid decrease in OUR and a slower decrease in P concentration followed by a slow decrease in OUR with a simultaneous slow increase in P concentration, see Fig 5.6(a and b).

The initial phase of P concentration decrease clearly is a continuation of the reaction in the aerobic reactor of the system, i.e. PHB utilization with some of the energy produced being used for polyP synthesis. By the end of the first phase the *Acinetobacter* has utilized all the PHB and completed its storage of polyP. The second phase is more interesting as it reflects complete endogenous behaviour. The slow decrease in OUR, and simultaneous slow increase in P concentration in the bulk solution in this phase was analysed as follows: Assuming that the decrease in OUR is proportional to the decrease in active mass, the rate of decrease in active mass was found to be 0,04 mg VASS/mgVASS/d (see Chapter 7 for details). With regard to the observed increase in P concentration this must be due to decrease in P stored by the sludge mass, and release of the P to the bulk solution. Relating the magnitude of P release to the stored P concentration indicates that the stored P decreased at a rate approximately equal to the rate of decrease of the active mass, i.e. the observed increase in P concentration probably was the release of stored P to the bulk solution due to *death* or endogenous mass loss of the *Acinetobacter* spp., *not* from polyP cleavage.

During the endogenous batch digestion test the *Acinetobacter* spp. are placed in an environment where no substrate, intracellular or extracellular, is present, but an external electron acceptor (oxygen) is present. In terms of the model the lack of substrate will switch off any synthesis pathways thereby removing ATP requirements for synthesis. Thus ATP is required only for cell maintenance. In cell maintenance generally a fraction of the cell protoplasm is utilized for energy (ATP) production –

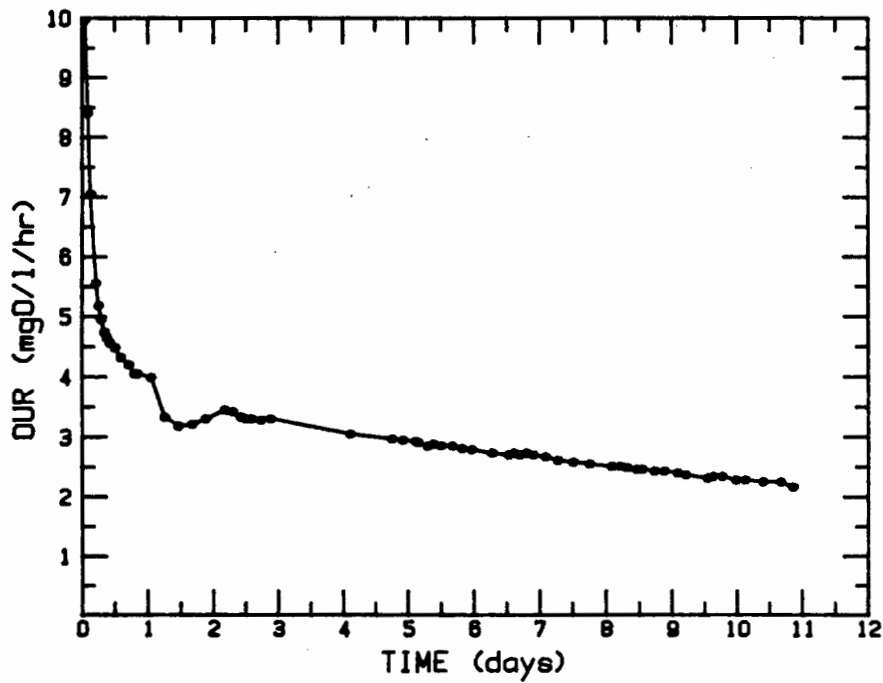


Fig 5.6(a): Oxygen utilization rate (OUR) with time in aerobic digestion batch test on mixed liquor drawn from aerobic zone of 3-stage modified Bardenpho enhanced culture system.

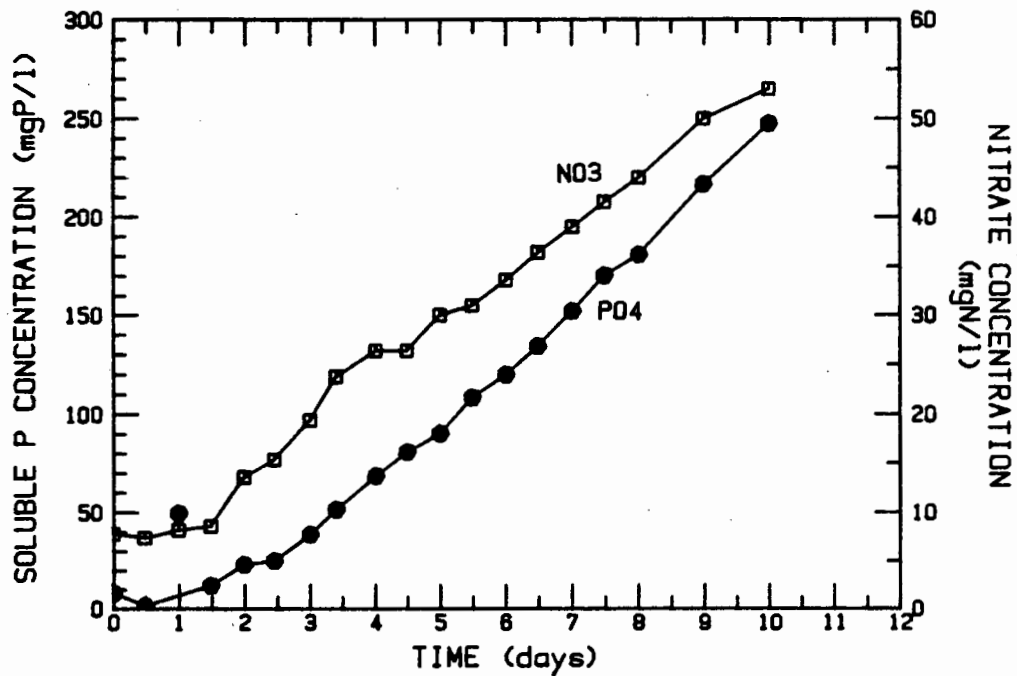


Fig 5.6(b): Phosphorus (P) and nitrate (NO_3) concentrations with time for aerobic digestion batch test in Fig 5.6(a).

giving rise to endogenous mass loss¹. This energy generation from cell protoplasm, together with the low ATP requirement, maintains the ATP/ADP ratio in the *Acinetobacter* spp. at a sufficiently high level to inhibit polyP degradation. Thus the loss of mass through endogenous action has associated with it the release of polyP stored by this mass to the bulk solution – this P release is *not* due to polyP cleavage for energy generation.

Now consider 'endogenous mass loss' under anaerobic conditions. If the *Acinetobacter* spp. are placed in an environment where no external electron acceptor is available they are unable to utilize the organic material released from cell death for energy generation. In this situation the cell maintenance requirements decrease the ATP/ADP ratio to a level that invokes polyP cleavage for energy production. The rate of P release under these conditions will be higher than under aerobic conditions because uncleaved polyP is released from cell death and P is released from polyP cleavage for maintenance energy. This has been observed when the aerated batch is allowed to become anaerobic².

Thus under endogenous conditions P is released under both aerobic and anaerobic conditions but at different rates. Because the P releases due to these two endogenous processes are not associated with PHB storage the released P cannot be regained. This will be so also under anoxic conditions (see below) and provides an explanation for the phenomenon of 'secondary release' sometimes reported on full-scale plants (Barnard, 1984).

¹Endogenous mass loss associated with maintenance can be viewed either as a decrease in the individual cell mass, or, as death of a fraction of the organism population releasing protoplasm as a substrate to the bulk liquid, in this fashion providing an energy source for the other cells for maintenance. In long sludge age systems, say 20 days, the mass of organisms receive only a small input of energy per day from the influent - this situation is not different from that where no substrate is fed. Under such starvation conditions it is unlikely that the cells synthesized would have 'spare' protoplasm for maintenance - protoplasm released from death (lysis) of weaker cells is the more likely source for maintenance energy. For an in-depth discussion, see Chapter 7.

²This situation may be complicated by the possibility that the protoplasm released from cell death might be converted to acetate whereupon it becomes available to the *Acinetobacter* for PHB storage by cleavage of polyP. However, this behaviour is unlikely because 'particulate' COD does not appear to be broken down to short chain fatty acids to any significant degree in anaerobic zones in these systems (Marais *et al*, 1983).

5.4 Anoxic environment

Under anoxic conditions oxidized nitrogen (e.g. nitrate) serves as the external electron acceptor. Lötter (1985) demonstrated that approximately 50 percent of *Acinetobacter* spp. strains isolated are capable of nitrate reduction. In Chapter 4 it was found that of the *Acinetobacter* spp. able to reduce nitrate the majority could only do so as far as nitrite, while only a minority could reduce nitrate (and nitrite) to nitrogen gas. Thus when explaining *Acinetobacter* spp. behaviour under anoxic conditions three "groups" of *Acinetobacter* must be considered; (i) those unable to utilize nitrate as an external electron acceptor, (ii) those able to reduce nitrate to nitrite only, and (iii) those able to reduce nitrate to nitrogen. It is of interest to speculate on the behaviour of these three *Acinetobacter* "groups" in two situations commonly encountered in biological excess P removal activated sludge systems, the (1) primary anoxic reactor and (2) the secondary anoxic reactor. In order to describe the behaviour as expeditiously as possible we accept that the reactors are plug flow, not completely mixed, this allows one to separate out the different phases of behaviour.

(1) Primary anoxic reactor: In the activated sludge systems, designed for nitrogen and excess P removal (modified Bardenpho and UCT systems, Marais *et al.*, 1983), mixed liquor passes from the anaerobic reactor to the primary anoxic reactor. In the anaerobic reactor short chain fatty acids (SCFA) have been sequestered by *Acinetobacter* spp. and stored as PHB at the expense of polyP. The *Acinetobacter* spp. entering the primary anoxic zone thus have a high intracellular substrate concentration (PHB), a low intracellular polyP concentration and a low extracellular substrate concentration in the bulk liquid. Consider the behaviour of each of the three *Acinetobacter* groups under such conditions:

(i) *Acinetobacter* spp. *unable to reduce nitrate*: This group of *Acinetobacter* spp. recognize the anoxic environment as anaerobic and behave accordingly. No oxidative phosphorylation takes place so that the ATP/ADP ratio decreases. This decrease stimulates polyP breakdown to produce energy (ATP) for cell maintenance. If SCFA leak from the anaerobic to the anoxic reactor, PHB is synthesized with concomitant P release (as in the anaerobic reactor). This group of *Acinetobacter* thus release P in the primary anoxic reactor.

(ii) *Acinetobacter* spp. *able to reduce nitrate to nitrite*: This group of

Acinetobacter spp. are able to produce ATP via oxidative phosphorylation by reducing nitrate to nitrite using stored PHB as substrate. However in the reduction of nitrate to nitrite only two molecules of ATP are generated per electron pair donated to nitrate compared with three ATP per electron pair for oxygen reduction (Payne, 1981). Thus the energy yield per electron pair in reducing nitrate to nitrite is much lower than in reducing oxygen. With the intracellular substrate available (PHB), this group of *Acinetobacter* probably will increase the ATP/ADP ratio sufficiently for polyP synthesis. However, due to the lower energy (ATP) yield per electron pair, the polyP synthesis per mass of substrate (PHB) utilized will be less under anoxic than under aerobic conditions. Furthermore, recognizing that both denitrification and aerobic oxidation are zero order reactions with respect to oxygen and nitrate concentrations, P uptake for these *Acinetobacter* spp. under anoxic conditions is likely to be at a slower rate than if the organisms were under aerobic conditions.

- (iii) *Acinetobacter* spp. able to reduce nitrate to nitrogen: This group of *Acinetobacter* spp. are able to produce ATP via oxidative phosphorylation by reducing nitrate to nitrogen using stored PHB as substrate. The energy yield per electron pair in reducing nitrate to nitrogen is equivalent to that in reducing nitrate to nitrite, i.e. 2ATP/electron pair. Thus this group of *Acinetobacter* spp. will exhibit a similar P behavioural pattern as the group able to reduce nitrate only to nitrite i.e. a relatively slow P uptake. However, the two *Acinetobacter* groups will differ in nitrate removal capacity because the electron accepting capacity in nitrate/nitrite reduction is only a quarter of that in the nitrate/nitrogen reduction. The *Acinetobacter* spp. reducing nitrate to nitrite only thus will reduce more nitrate per electron pair (equivalent to COD utilized) than the *Acinetobacter* spp. reducing nitrate to nitrogen.

The combined behaviour of the three *Acinetobacter* spp. groups gives rise to the observed overall behaviour. The observed behaviour is likely to be a rapid decrease in nitrate, an associated decrease in PHB and either a slow increase or decrease in P concentration depending on the relative fractions of *Acinetobacter* spp. present.

The speculated interaction between the three groups of *Acinetobacter* can account for the apparently contradictory observations of behaviour in primary anoxic zones; that of P release or uptake.

- (2) Secondary anoxic reactors A secondary anoxic reactor is found in 5-stage modified Bardenpho systems, the reactor being placed immediately downstream of the aeration reactor. In the main aeration reactor the stored PHB is likely to have been very nearly or completely utilized and polyP storage similarly completed. In terms of the model on entry of mixed liquor to the secondary anoxic zone: Those *Acinetobacter* unable to reduce nitrate will respond as if the reactor is anaerobic, i.e. P release due to endogenous death and polyP cleavage for maintenance energy as in the section on endogenous "mass loss" under anaerobic conditions; those *Acinetobacter* spp. capable of reducing nitrate to nitrite and those capable of reducing nitrate to nitrogen gas will behave in the same fashion as *Acinetobacter* spp. in the primary anoxic reactor provided PHB is available. Should PHB be reduced to zero, these *Acinetobacter* spp. will behave as under aerobic endogenous conditions, that is, the fraction of their mass released on death is used to supply ATP for cell maintenance purposes and the polyP associated with the metabolized protoplasm mass is released giving rise to an observed P release.

In the secondary anoxic reactor if nitrate becomes depleted, i.e. an anaerobic state, all three groups of *Acinetobacter* spp. will behave as described for anaerobic endogenous conditions.

We can now apply the above behavioural patterns to explain observed system response:

- (1) In the 3-stage modified Bardenpho or UCT systems with completely mixed reactors, in the aerobic reactor, release of P due to endogenous respiration effects is virtually certain to be swamped by polyP accumulation from PHB metabolism, and a net removal of P from the liquid will be observed.
- (2) In the 5-stage modified Bardenpho system, i.e. one that includes a secondary anoxic zone, the behaviour in the secondary anoxic zone will be governed to a large extent by the mass of PHB still stored in the sludge entering that zone, and the concentration of nitrate. If the nitrate and PHB concentrations are adequate then polyP storage will continue and probably will exceed P

release due to endogenous metabolism and other effects. If stored PHB is low the polyP storage may be either more, equal or less than the release phenomena and correspondingly, P uptake, no change, or P release will be observed. If there is no stored PHB then certainly P release will be observed. This will also be the case when no nitrate is present, whether PHB is present or not. P release associated with energy production for maintenance cannot be taken up subsequently on aeration. The behaviour deduced above has been reported: Barnard (1984) has observed P release in some secondary anoxic reactors of full-scale modified Bardenpho plants, and that the released P was not taken up in the subsequent reaeration reactor. This phenomenon he termed 'secondary' release. It would appear that endogenous ('secondary') P release takes place throughout the system, its manifestation usually being swamped by other biological phosphorus processes. This implies that the longer the sludge age the greater the release, consequently, for the same substrate mass utilized by the polyP organisms the net removal of P from the system should diminish as the sludge age increases. Observations on enhanced *Acinetobacter* spp. activated sludge systems appear to support this (Wentzel *et al.*, 1988c), see Chapter 7.

Space does not allow detailed discussion of the many different situations that can arise in the main aeration, secondary anoxic and reaeration reactors.

5.4 Nutrient limitation

If any essential nutrient becomes limiting and a suitable carbon substrate is available, polyP and PHB accumulation is likely to occur. This is because the synthesis pathways will be inhibited by the limiting nutrient, this inhibition decreasing the ATP and substrate requirements for synthesis. The TCA cycle will not be inhibited by nutrient deficiency so that the NADH/NAD ratio will be maintained at a high level and oxidative ATP production will continue elevating the ATP/ADP ratio. The high ATP/ADP will stimulate polyP accumulation, while the high substrate concentration and NADH/NAD ratio will stimulate PHB accumulation (with the proviso that the substrate concentration is sufficiently high). PolyP accumulation under limitation of the nutrients sulphur and nitrogen has been observed, the so-called 'luxury uptake', (Harold, 1966) and appears to find explanation in terms of this model. If however the limiting nutrient is one of the cations required to stabilize polyP charge, i.e. Mg^{2+} or K^+ , then synthesized polyP will be unstable and no polyP accumulation will take place. This behaviour has been

explicitly verified in enhanced *Acinetobacter* spp. activated sludge systems, see Chapter 6.

6. CONCLUSIONS

Acinetobacter spp. are able to compete successfully with other organisms for substrate in completely aerobic activated sludge systems. In such systems *Acinetobacter* spp. are able to metabolize glucose via the Entner-Doudoroff pathway. When an anaerobic reactor is introduced into the system the anaerobic reactor selects an assemblage of facultative organisms able to ferment sugars to short chain fatty acids (SCFA) via the glycolytic (Embden-Meyerhof) pathway. *Acinetobacter* spp. do not possess the glycolytic pathway and thus are not able to produce energy for survival in the anaerobic zone by e.g. glucose fermentation. Consequently the *Acinetobacter* spp. would be at a distinct disadvantage in the anaerobic/aerobic system due to this lack of the glycolytic pathway and would be outcompeted by the facultative organisms in such a system. Without some mechanism of competitive compensation they would disappear from the anaerobic/aerobic activated sludge system. This competitive compensation is the ability to store polyP and PHB. During the anaerobic state polyP serves as an energy source for maintenance of cell functions and PHB synthesis. PHB synthesis in the anaerobic phase effectively removes the *Acinetobacter* from competition for substrate enabling the organism to withstand the selective pressures of the anaerobic/aerobic sequence.

The biochemical model presented here describes the pathways whereby *Acinetobacter* spp. accumulate and degrade polyP and PHB, and how these pathways are regulated. Identification of the regulatory system allows one to predict the response of the organism under a variety of imposed conditions. Principally regulation appears to be via the ATP/ADP and NADH/NAD ratios. These ratios are affected by the substrate concentration, intracellular or extracellular (PHB and, say, acetate respectively), and the presence or absence of external electron acceptors (aerobic, anoxic or anaerobic). In terms of these parameters it appears to be possible to explain the observed behaviour of biological excess phosphorus removal as observed in plants and other situations. These are briefly summarized below:

(1) Under aerobic conditions:

- (i) If acetate concentration is high both PHB and polyP storage takes place; this is observed in pure culture tests.

- (ii) If acetate concentration is relatively low *Acinetobacter* spp. grows without PHB or polyP accumulation; this situation is relevant to completely aerobic activated sludge systems.
- (iii) If acetate concentration is very low or zero, but stored PHB is present, polyP accumulation will take place; this situation is relevant to the main aeration basin of anaerobic/anoxic/aerobic systems (modified Bardenpho or UCT).
- (iv) If neither acetate nor PHB is present, maintenance energy is obtained by utilization of substrate generated by the death of *Acinetobacter* spp. (endogenous mass loss); polyP is released apparently uncleaved to the bulk solution proportionally to the protoplasm mass of organisms that died; this situation might be observed explicitly in the reaeration zone (and in the secondary anoxic zone if nitrate is present – see 3(ii) below) and is one component of the so-called secondary release. Speculatively it would appear that death of *Acinetobacter* and associated release of uncleaved polyP takes place throughout the system, i.e. whether acetate is available or not; but because of its low rate its manifestation is swamped by other biological phosphate processes. This secondary P release is never associated with any form of substrate storage and will not give rise to any form of subsequent P uptake.

(2) Under anaerobic conditions:

- (i) If acetate is present, PHB is stored by cleaving stored polyP. Also, maintenance energy is obtained by cleavage of stored polyP.
- (ii) If acetate is not present, irrespective of whether PHB is present or not, maintenance energy is obtained by cleavage of stored polyP. This is another component of secondary release and again is not associated with subsequent P uptake.

(3) Under anoxic conditions (nitrate present):

- (i) If PHB is present but acetate is not, as in the primary anoxic zone,

two situations can apply: *Acinetobacter* not able to use nitrate react as in 2(ii); *Acinetobacter* that can use nitrate react as in 1(iii). Hence a release and uptake of P takes place simultaneously giving rise to a net P release or uptake, depending on the relative concentrations of organisms and the rates of reaction.

- (ii) If neither PHB nor acetate is present, as in the secondary anoxic zone, then: *Acinetobacter* not able to use nitrate react as in 2(ii); *Acinetobacter* that can use nitrate react as in 1(iv). However in the secondary anoxic zone if no nitrate is present, maintenance energy cannot be obtained by oxidation of the protoplasm released due to death; instead it is obtained by cleavage of polyP giving rise to secondary release as in 2(ii). When no nitrate is present the protoplasm released due to death may be converted to acetate or other SCFA by non-polyP facultative organisms, so that P release due to PHB formation can take place and subsequently on aeration give rise to an uptake of P, usually limited. This behaviour has been observed in reaeration zones on occasion.

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CHAPTER 6

ENHANCED POLYP ORGANISM CULTURES – SYSTEM DEVELOPMENT

1. INTRODUCTION

In Chapter 3 it was concluded that P uptake kinetics could not be formulated without explicitly incorporating the growth and death (or endogenous mass loss) characteristics of the polyP organisms. These characteristics could not be isolated in mixed culture activated sludge systems for reason that the responses of the non-polyP heterotrophic organisms either obscured or swamped out those of the polyP organisms. To overcome this problem it was suggested that elucidation of the characteristics of the organisms would require the development of cultures in which the polyP organisms dominate.

One approach to obtain such a culture is to employ pure culture techniques. This would demand positive selection of the organism to be cultured. In the literature an organism group widely implicated in biological excess P removal is the genus *Acinetobacter*. Numerous pure culture studies have been conducted to ascertain the growth and death characteristics of strains belonging to this genus (Abbott *et al.*, 1973; Abbott *et al.*, 1974; Ensley and Finnerty, 1980; Du Preez, 1980; Du Preez *et al.*, 1981 and van Groenestijn and Deinema, 1985). These studies all were conducted using pure cultures of specific *Acinetobacter* strains or species grown in aerobic chemostats, with very short sludge ages (up to one day) and with acetate or ethanol as influent substrate, but at high COD concentrations. The results of the studies show remarkable similarity, summarized in Table 6.1.

Referring to Table 6.1, the stoichiometric yield coefficients for acetate (0,43 gcells/gCOD) and for ethanol (0,40 gcells/gCOD) compare favourably with values reported for 'normal' activated sludge organisms (0,45 gVSS/gCOD, Marais and Ekama, 1976). However, the respective specific kinetic rate constants for growth and decay differ significantly from those reported for 'normal' sludges in activated sludge systems: The maximum specific growth rate constant ($\hat{\mu}$) for *Acinetobacter*, with ethanol and with acetate, is extremely high (14–30/d) compared with that for 'normal' activated sludge (2 to 4/d, Ekama *et al.*, 1986). Similarly the specific endogenous mass loss rate constant is exceptionally large (1-5/d) compared with that for 'normal' activated sludge (0,24/d, Marais and Ekama, 1976). The high specific endogenous mass loss rate of the *Acinetobacter* in particular, is directly in contradiction to the conclusions in Chapter 3, that the rate for polyP organisms

Table 6.1: Growth and death characteristics of various *Acinetobacter* strains (species) measured in pure cultures grown in aerobic chemostats.

Research Group	Organism	Substrate	Yield (gcells /gCOD)	Max. spec. growth rate (/d)	Spec. end. mass loss rate (/d)
Abbott et al. (1973, 1974)	<i>Acinetobacter calcoaceticus</i>	ethanol	0,38	17	2
Ensley and Finnerty (1980)	<i>Acinetobacter</i> spp.	acetate	-	14	2
Du Preez et al. (1981)	<i>Acinetobacter calcoaceticus</i>	ethanol, acetate and ethanol/acetate mixture	0,43	4-30	1-5
van Groenestijn and Deinema (1985)	<i>Acinetobacter</i> strain A102	acetate	0,40	17	1

*Specific endogenous mass loss rate constant calculated (from data supplied in the respective papers) using the Monod-Herbert approach (synthesis-endogenous respiration) see Marais and Ekama, 1976.) instead of the Monod-Pirt approach (synthesis-maintenance energy) used in the papers.

appears to be very much *smaller* than that for 'normal' heterotrophs. These differences indicate that either two different polyP organism types were present in the pure and mixed cultures respectively, or, the same organism type was present but developed divergent behavioural characteristics when exposed to the two different sets of environmental conditions (*Acinetobacter* pure cultures were grown aerobically at short sludge ages, whereas in the mixed cultures these organisms were developed in anaerobic/anoxic/aerobic activated sludge systems at long sludge ages). The influence of the environmental conditions on the same species could be checked by growing the selected polyP species in pure culture in a system of interlinked chemostats simulating the same configuration as that of the modified Bardenpho or UCT excess P removal plants and comparing the response with that observed in the aerobic pure culture studies. However, a simpler approach was devised, to grow an *enhanced* culture of polyP organisms. By enhanced culture is meant: The development of a polyP organism culture by selecting a substrate and set of environmental conditions that favour polyP organisms so that they become the dominant primary organism and their behaviour dominates the culture response. Growth of competing normal heterotrophic primary organisms will be curtailed naturally but not necessarily terminated, neither will predation by higher organisms and other interactive effects be positively excluded. Also, a strain (or strains) of polyP organism will be naturally selected which may differ from that artificially selected and grown in pure culture in a chemostat system. This chapter reports on the development of enhanced polyP organism cultures.

2. CONDITIONS FOR ENHANCED CULTURE DEVELOPMENT

To identify the conditions likely to produce an enhanced culture of polyP organisms, *Acinetobacter* spp. were accepted as organisms typical of the polyP group. In Chapter 4 *Acinetobacter* spp. were shown to be present abundantly in both aerobic and anaerobic/anoxic/aerobic activated sludge systems. It was concluded that these organisms have the propensity to exhibit biological excess P uptake, but this is invoked only with the appropriate substrate and environmental conditions. In Chapter 5 a biochemical model was put forward in terms of which sets of conditions that give rise to biological excess P removal processes can be identified. One set is to subject the organism mass to an anaerobic/aerobic sequence with short chain fatty acids fed to the anaerobic phase, conditions present in the Phoredox, modified Bardenpho and UCT systems with short chain fatty acids as influent. PolyP accumulating organisms sequester the short chain fatty acids (e.g. acetate) in the anaerobic phase for their exclusive use in the subsequent aerobic phase. Hence, with acetate as the sole substrate fed to the anaerobic phase of an anaerobic/aerobic

sequence, the acetate would be available only to the polyP organisms, providing a situation favourable for these organisms, enabling them to become dominant. Furthermore, according to the biochemical model these conditions would stimulate the processes involved in biological excess P removal. Based on these considerations a study into the development of enhanced cultures of polyP organisms was inaugurated.

3. SYSTEM DEVELOPMENT

When investigations into the development of an enhanced culture of polyP accumulating organisms commenced in February 1984, little information was available in the literature on the protocol for such an investigation. As a consequence, initial experimentation was almost totally of a trial and error nature. It may be useful to other investigators, therefore, to relate briefly the evolution of the procedures, the dead ends and the byways encountered, to obtain an enhanced culture.

3.1 Phoredox system 1

Experiments were initiated using an anaerobic/aerobic (Phoredox) configuration operated at 10 days sludge age. The system set up is shown in Fig 6.1. As a control, a single completely mixed aerobic activated sludge reactor was set up, having the same volume and sludge age, and receiving the same substrate and volume of influent feed per day. Both systems were operated at 20°C, as were all subsequent systems. To eliminate confounding effects of nitrification and denitrification, nitrification was suppressed by periodic addition of thiourea to both systems as follows: Whenever the effluent TKN commenced to decrease, 20 mg thiourea per litre mixed liquor was added to the respective reactors.

To start up, both systems were inoculated with mixed liquor from a laboratory scale UCT system operated at 20 days sludge age on unsettled municipal wastewater. To acclimatize the organism mass to acetate, the systems were fed with a mixture of 500 mgCOD/l unsettled municipal wastewater from Mitchell's Plain, Cape Town, plus 500 mgCOD/l sodium acetate, i.e. 1 000 mgCOD/l. To counter the possibility of deficiency in nutrient requirements, additional inorganic *macro*-nutrients were added according to the recipes of Abbott (1973) and Du Preez (1980) as set out in Table 6.2. In their nutrient recipes the magnesium concentrations were much lower than those given by Fuhs and Chen (1975). However, Fuhs and Chen stated that magnesium was unlikely to be a limiting nutrient as it acted as cofactor in biological

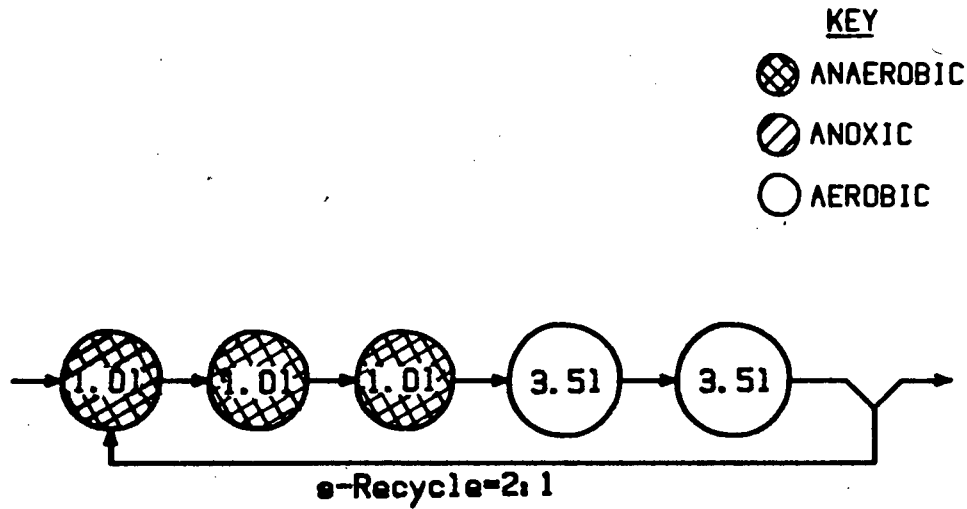


Fig 6.1: Schematic layout of Phoredox (A/O) system; reactor volumes in litres, sludge age 10 days and influent flow rate 7,5ℓ/d.

Table 6.2: Inorganic macro-nutrients added per 1 000 mgCOD sodium acetate to influent consisting of a mixture of unsettled municipal waste water and sodium acetate (adapted from Du Preez, 1980).

Chemical	mg added/1000 mgCOD sodium acetate	
	Compound	Element
NH_4Cl	162,2	42,4 N
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	40	3,9 Mg
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	5,34	1,45 Ca
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	5,34	1,32 Mn
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0,197	0,0396 Fe
KH_2PO_4	52,66	(62,91 K
K_2HPO_4	106,66	(31,01 P

reactions and hence was required only in low concentrations. Thus the magnesium and other macro-nutrient concentrations given by Abbott (1973) and Du Preez (1980) were used as a starting point. The *micro*-nutrients suggested by Du Preez were not added as it was considered that these would be adequately supplied by the wastewater fraction and the tap water used in dilution. The characteristics of the unsettled wastewater fraction were approximately as shown in Table 6.3(a) and those of the tap dilution water approximately as shown in Table 6.3(b).

The systems were operated for two weeks, whereupon both had approximately the same sludge concentrations. The mixed liquor in the Phoredox system settled well, but that in the aerobic system bulked excessively. For the aerobic system the steady state P removal was around 0,005 mgP/mg influent COD which conforms to the normal P requirements of non-polyP heterotrophs at 10 days sludge age. For the Phoredox system the steady state average P removal was 15 mgP/l (i.e. 0,015 mgP/mg influent COD) and the average P release was 60 mgP/l influent (0,06 mgP/mg influent COD). This removal was *less* than that obtained on a UCT system operated at 20 days sludge age with 1 000 mgCOD/l unsettled municipal wastewater (removal 20 mgP/l i.e. 0,02 mgP/mg influent COD) even though the substrate fraction favourable to the polyP organisms was three times higher for the Phoredox (500 mgCOD/l acetate plus 100 mgCOD/l S_{bs}) than for the UCT system (200 mgCOD/l S_{bs}). No assignable cause for this lower P removal could be discerned, except possibly a deficiency in the inorganic nutrients. In an endeavour to gain further information on the nutrient requirements, the influent was changed to pure acetate at 1 000 mgCOD/l and, using the nutrient recipe of Du Preez (1980) as a basis, fractional nutrient concentrations relative to the acetate were increased to four times those suggested by Du Preez (1980). It was presumed that the concentrations reduced the likelihood that the macro-nutrients possibly could be the limiting factor. The augmented nutrient concentrations are given in Table 6.4. Micro-nutrients were not added as the elements specified by Du Preez (1980) appeared to be in adequate concentrations in the tap water [see Table 6.3(b)]. Immediately the new substrate was fed to the Phoredox system, the release in the anaerobic zone commenced to increase, so also the removal. An unexpected feature noted was that the pH in the aerobic reactor increased sharply relative to that in the anaerobic reactor, 8,7 as against 7,2. To counter this, 0,3 ml concentrated HCl per litre influent was added to the influent batch. This controlled the pH in the aerobic reactors to about 8,4, but the pH in the anaerobic reactor now fell to about 6,0. To avoid the reduction in pH in the anaerobic reactor, the point of addition of HCl was changed to the aerobic reactor. By doing this, the pH was maintained at $\pm 7,4$ in the aerobic and $\pm 7,1$ in

Table 6.3(a): Characteristics of unsettled municipal wastewater.

Component	mg/1 000 mgCOD
Total COD (S_{ti})	1000 COD
Readily biodegradable COD (S_{bsi})	≈ 200 COD
Unbiodegradable soluble COD	≈ 100 COD
Unbiodegradable particulate COD	≈ 80 COD
TKN	≈ 100 N
P	≈ 18 P
Mg	≈ 7,00 Mg
Ca	≈ 38 Ca
Mn	≈ 0,12 Mn
Fe	≈ 0,80 Fe
Zn	≈ 0,30 Zn
Cu	≈ 0,12 Cu
Co	≈ 0,02 Co
Mo	≈ 0,06 Mo
Al	≈ 1,80 Al
Ni	≈ 0,04 Ni

Table 6.3(b): Selected characteristics of tap water.

Component	Approximate concentration (mg/l)
Mg	2,4
Ca	20
Mn	0,02
Fe	0,1
Zn	0,15
Cu	0,04
Co	NIL
Mo	NIL
Al	1,7
Ni	NIL

the anaerobic reactors. (Maintaining the pH between 7,0 and 7,5 minimized the possibility of calcium phosphate precipitation.) This method of maintaining the pH was used subsequently in all the systems operated. [The loss of protons (H^+) in the aerobic zone of biological excess P removal systems has been explained biochemically in Chapter 5].

Operating the Phoredox system in the fashion described above, within about 10 days the release had increased to 130 mgP/l influent (0,13 mgP/mg influent COD), and the removal to 20 mgP/l (0,02 mgP/mg influent COD). However, the sludge settleability in the Phoredox system now deteriorated, to such an extent that the settler overflowed and the system had to be abandoned. Microscopic examination of the sludge showed that the bulking was due to the massive formation of extracellular polysaccharide slimes. Such slime formation often is due to some nutrient deficiency. Warskow and Juni (1972) had observed that, in pure culture studies, one strain of *Acinetobacter* failed to grow in an acetate-mineral medium unless a trace of yeast extract was added. Accordingly in the next series of experiments (Phoredox system 2), and all future experiments, yeast extract was added to the influent.

3.2 Phoredox system 2

A Phoredox system again was set up, as shown in Fig 6.2. It was hypothesized that perhaps the sludge age had been too low and this was set to 20 days. Again thiourea was added periodically to inhibit nitrification. The total influent COD was set at 500 mgCOD/l to obtain a more direct comparison with other excess P removal systems operated in the laboratory on unsettled municipal wastewater. The acetate fraction in the influent was increased incrementally, keeping the total COD constant at 500 mg/l, i.e. if, for example, 100 mg/l COD acetate was included, the sewage fraction was reduced to 400 mgCOD/l. Yeast extract was added in the proportion, 1 mg extract for every 100 mgCOD of sodium acetate. With each increment in acetate the system was run until apparent steady state behaviour was attained. In this fashion the acetate COD concentrations were set sequentially at 50, 100 and 200 mg/l plus yeast extract. In order that the onset of nutrient deficiency could be determined, no inorganic macro- or micro-nutrients were added to the influent (except for NH_4Cl and K_2HPO_4 to give the desired N and P influent concentrations respectively). An aerobic control unit again was set up, receiving the same influent and operated at the same sludge age as the anaerobic/aerobic unit.

In the Phoredox system, the P removal achieved with the increased fractions of acetate in the influent is shown in Fig 6.3. For all the acetate fractions the P

Table 6.4: Influent inorganic nutrient concentrations added to influent of 1 000 mgCOD sodium acetate, adapted from Du Preez (1980).

Chemical	mg added/1 000 mgCOD sodium acetate	
	Compound	Element
NH_4Cl	153	40 N
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	200	19,5 Mg
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0,7	0,14 Fe
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	20	5,44 Ca
KH_2PO_4	98,75	118 K
K_2HPO_4	200	58,14 P

KEY

⊗ ANAEROBIC

⊘ ANOXIC

○ AEROBIC

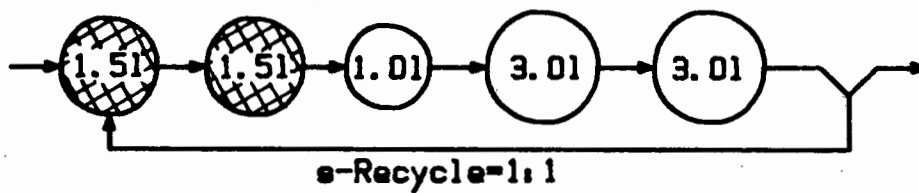


Fig 6.2: Schematic layout of Phoredox (A/O) system; reactor volumes in litres, sludge age 20 days and influent flow rate 15l/d.

removal hardly changed and averaged at about 11 mgP/l (i.e. 0,022 mgP/mg influent COD). In the aerobic control unit P removal remained at about 0,004 mgP/mg influent COD. Settleability in the anaerobic/aerobic unit now was excellent with no slime development indicating that the yeast extract served some essential function in the system. The aerobic system continued to bulk, but the bulking now was due to filamentous organisms, not slime formation.

In seeking a reason for the insensitivity of P removal to the acetate feed it was hypothesized that the acetate may not be a good carbon source for metabolic synthesis and, accordingly, an influent consisting of 250 mgCOD/l sewage, 125 mgCOD/l acetate, 125 mgCOD/l lactate was tried for both systems - P removal remained at approximately 10 mgP/l (i.e. 0,02 mgP/mg influent COD) for the anaerobic/aerobic unit and 0,004 mgP/mg influent COD for the aerobic control. The acetate substrate was then abandoned and an influent of 250 mgCOD/l lactate and 250 mgCOD/l sewage was tried. The Phoredox system's release declined slightly, from 60 mgP/l influent (0,12 mgP/mg influent COD) to 50 mgP/l influent (0,10 mgP/mg influent COD), but the removal remained at 10 mgP/l (0,02 mgP/mg influent COD). Evidently lactate addition conferred no advantage on the system. The influent feed was then changed to 250 mgCOD/l sewage and 250 mgCOD/l acetate, but the removal continued to remain at about 10 mgP/l (0,02 mgP/mg influent COD). The aerobic control unit continued to remove 0,005 mgP/mg influent COD.

During these investigations it was noticed that every time thiourea was added to the system, the removal of P in both the anaerobic/aerobic and the aerobic units showed a decline. It was decided therefore to reduce the frequency of thiourea addition, but this did not improve the mean removal. It was thereupon decided to abandon thiourea addition and to reduce the nitrate (NO_3^-) by introducing a primary anoxic reactor with an a-recycle from the last aerobic zone to the primary anoxic reactor (i.e. setting up a laboratory scale 3-stage modified Bardenpho system), see Fig 6.4.

3.3 Modified Bardenpho system

For this system, the influent was maintained at 250 mgCOD/l acetate and 250 mg/l sewage. The release fluctuated around 100 mgP/l, but the average removal continued to remain at 10 mgP/l (0,02 mgP/mg influent COD).

At this juncture an analysis of the organisms present in the mixed liquor from the Bardenpho system was undertaken by Laboratory and Technical Services Branch of

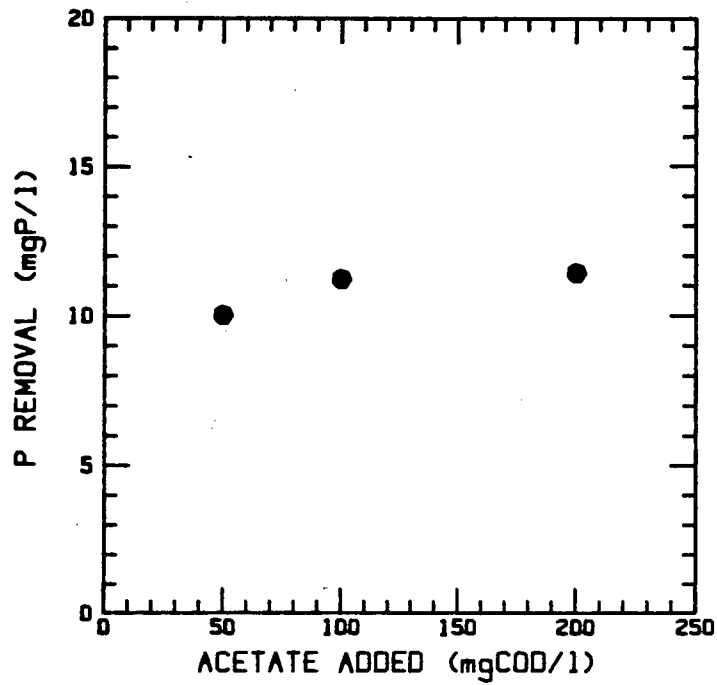


Fig 6.3: Phosphorus (P) removal versus acetate added to the influent to Phoredox system (Fig 6.2) when there is a deficiency of magnesium. Total COD is 500 mg/l.

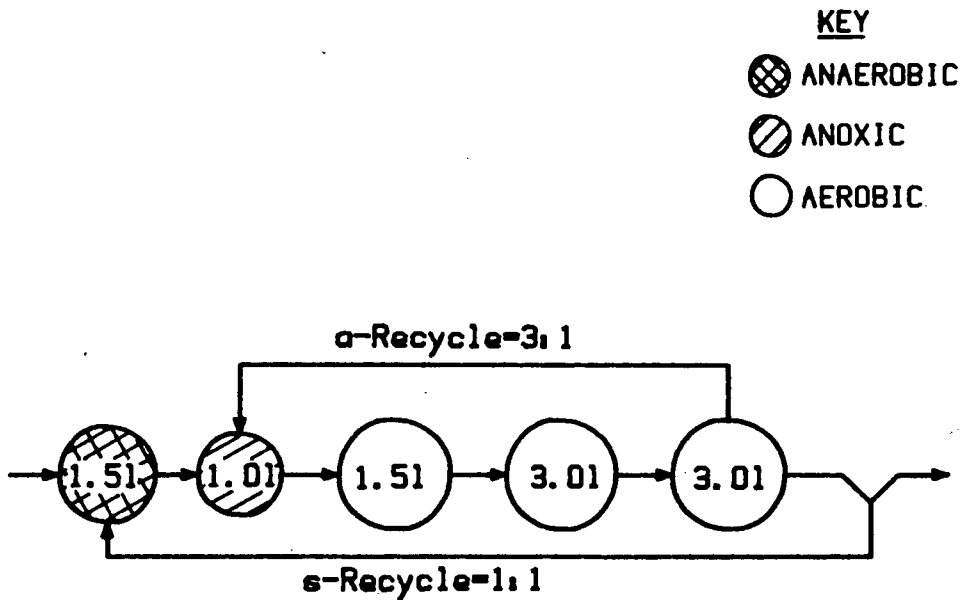


Fig 6.4: Schematic layout of 3-stage modified Bardenpho system; reactor volumes in litres, sludge age 20 days and influent flow rate 15 l/d.

the City of Johannesburg using the Analytical Profile Index (API) procedure (Analytlab Products, 1977). This showed that, of the organisms cultured aerobically, 90 percent were *Acinetobacter* spp. It seemed clear, therefore, that there was not a nutrient deficiency insofar as growth of this species was concerned. This, together with the remarkable constancy of the P removal at about 10 mgP/l (0,02 mgP/mg influent COD), irrespective of the magnitude of the release or the type of substrate added, eventually forced the conclusion that there might be a deficiency in some chemical that affected the P uptake and excess P removal. Biochemically the counter-ion for ATP stabilization is Mg^{2+} and it was hypothesized that, for the stabilization of the polyP chains, the counter-ion similarly was Mg^{2+} . Accordingly, with influent remaining at 250 mgCOD/l acetate and 250 mgCOD/l sewage, the influent was augmented by 264 mg $MgCl_2 \cdot 6H_2O$ /l (30 mg Mg^{2+} /l). Immediately the removal increased from 10 to 18 mgP/l and the release from 100 to 120 mgP/l influent. To confirm that the Mg^{2+} was the prime deficient nutrient the Mg^{2+} was omitted – this caused a precipitous drop in the P removal to 12 mgP/l. It was concluded therefore that the 250 mgCOD/l acetate-250 mgCOD/l sewage mixture contained inadequate Mg^{2+} . An examination on an ionic charge basis indicated that for every two P removed two negative charges had to be neutralized; if the charges were neutralized by magnesium, one Mg^{2+} would be required as a counter-ion for every two P removed. Accordingly, using this ratio, Mg^{2+} was added to the influent, sufficient for a P removal of 30 mgP/l. A steady state was attained with P removal of 25 mgP/l (0,05 mgP/mg influent COD) and with P release of 110-130 mgP/l influent (0,24 mgP/mg influent COD). This steady state was readily maintained.

Once the importance of magnesium was established, the acetate fraction was increased to 300 mgCOD/l, the sewage fraction correspondingly decreased to 200 mgCOD/l, the magnesium was increased proportionally, also the yeast extract, but no other nutrient was augmented. However, no apparent change was observed in the magnitude of P release and P removal, from that obtained with lower acetate concentration. Moreover, the system response now was unstable, exhibiting cyclic fluctuations. It was hypothesized that such fluctuations could be the result of some further nutrient limitation. The element calcium (Ca^{2+}) functions as a cofactor in numerous enzyme reactions in biological systems and also has been implicated as another cation stabilizing the polyP chain (Buchan, 1981). In consequence, it was decided to ensure adequate calcium by increasing calcium in the influent by 10 mg Ca^{2+} /l. Immediately the P release, uptake and removal exhibited an increase, P removal stabilized at about 30 mgP/l (i.e. 0,06 mgP/mg influent COD) and release at 160-180 mgP/l influent (i.e. \approx 0,34 mgP/mg influent COD). Evidently the Ca^{2+}

ion was an essential macro-nutrient in polyP metabolism.

At the beginning of June 1985, and at intervals thereafter, the acetate fraction was progressively increased to 350, 400 and 500 mgCOD/l, the sewage fraction being decreased correspondingly to 150, 100 and zero mgCOD/l. The macro-nutrients, Mg^{2+} and Ca^{2+} , and the yeast extract were increased proportionally to the acetate in accordance with the proportions set out in Table 6.5. Phosphorus (P) was added to the influent in sufficient concentration so as to ensure that the effluent always contained P. The P was added as K_2HPO_4 . With this addition K did not become limiting (on a molar basis two K were added for every P). However, batch tests conducted on sludge samples drawn from the enhanced cultures indicated that K release and uptake was linearly associated with P release and uptake, 0,3 moles K/mole P. Similarly in the batch tests, Mg release and uptake was linearly associated with P release and uptake, 0,26 moles Mg/mole P. In contrast, very little Ca release and uptake was associated with the P release and uptake, 0,05 moles Ca/mole P. Nitrogen was added as ammonia to maintain an influent TKN/COD ratio of about 0,06 mgN/mgCOD.

When the acetate fraction increased to 400 mgCOD/l and above, the possibility of inadequacy of micro-nutrients was raised. In pure culture studies Du Preez (1980) described a micro-nutrient solution for growth of *Acinetobacter*, a polyP organism. As a *precautionary* measure, a similar micro-nutrient solution was added to the influent to supply elements essential to biological function. The concentrations of micro-nutrients added to the influent, adapted from Du Preez (1980), are given in Table 6.5. The data obtained, on P release and uptake (and hence P removal) versus acetate added in this series of tests, are shown plotted in Fig 6.5(a and b) – the responses are near linear.

After the system with acetate as the sole carbon and energy source had been operating stably for about a month the sludge was again analysed using the Analytical Profile Index (API) method. The analysis indicated that of the organisms cultured aerobically, virtually 100 percent were *Acinetobacter* spp. - an enhanced culture of the polyP organisms had been achieved.

3.4 UCT system

Concomitant with the Bardenpho system, a UCT system was set up for development of another enhanced culture of polyP organisms in May 1985. A UCT system was selected as it reduces the nitrate recycled to the anaerobic reactor. Furthermore, a

Table 6.5: Influent macro- and micro-nutrient added per 1 000 mgCOD sodium acetate to the influent, adapted from Du Preez (1980).

Chemical	mg added/1 000 mgCOD sodium acetate	
	Compound	Element
MgCl ₂ ·6H ₂ O CaCl ₂ ·2H ₂ O Yeast extract	Macro-nutrients	
	468	56 Mg
	117	32 Ca
FeSO ₄ ·7H ₂ O ZnSO ₄ ·7H ₂ O MnSO ₄ CuSO ₄ ·5H ₂ O CoCl ₂ ·6H ₂ O Na ₂ MoO ₄ ·2H ₂ O H ₃ BO ₃ KI	10	
	Micro-nutrients	
	5,25	1,05 Fe
	1,5	0,34 Zn
	1,5	0,55 Mn
	0,3	0,076 Cu
	0,3	0,074 Co
	0,15	0,059 Mo
	0,3	0,052 B
	0,075	0,057 I

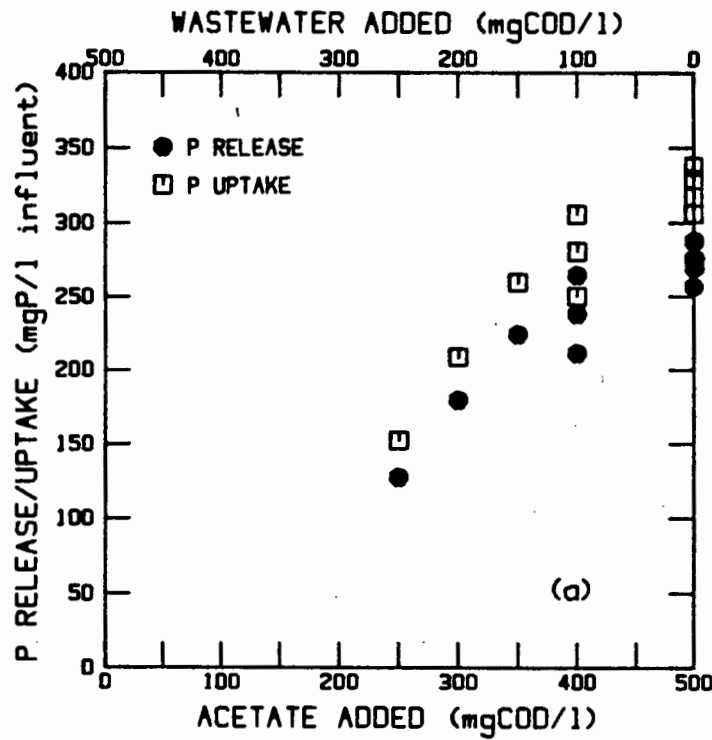


Fig 6.5(a):

Total soluble phosphorus (P) release and uptake in 3-stage modified Bardenpho system (Figs 6.4 and 6.8) with increasing concentration of acetate in a total influent COD of 500 g/l (wastewater + acetate).

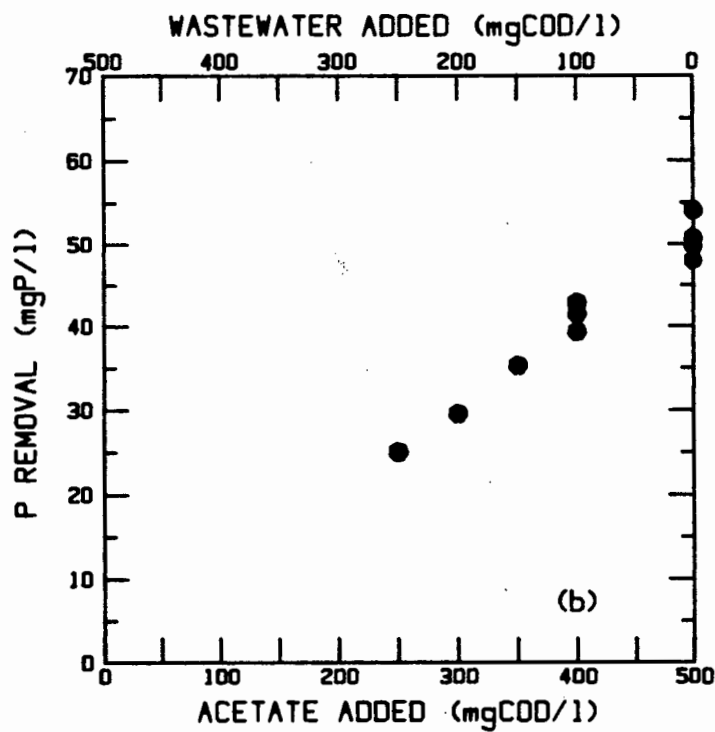


Fig 6.5(b):

Total soluble phosphorus (P) removal for data in Fig 6.5(a).

comparison of UCT system behaviour with that of the modified Bardenpho system would be possible. To obtain some information on the effect of sludge age on polyP organism behaviour, it was decided to operate the UCT system at a sludge age of 10 days, as opposed to the modified Bardenpho system sludge age of 20 days. The layout of the UCT system is given in Fig 6.6. Following the success of operation of the modified Bardenpho system with a mixture of 250 mgCOD/l acetate, 250 mgCOD/l sewage, yeast extract and mineral nutrients, and to acclimatize the organisms to acetate substrate, it was decided to start the UCT system with this influent mixture. Waste mixed liquor from the enhanced culture modified Bardenpho system, together with mixed liquor from a laboratory scale modified UCT activated sludge system treating raw sewage, was used to inoculate the system. The acetate fraction of the influent again was increased incrementally, from 250 to 350 and finally to 500 mgCOD/l, with a corresponding decrease in the sewage fraction - 250, 150 and 0 mgCOD/l. Macro- and micro-nutrients and yeast extract were added to the influent in the proportions set out in Table 6.5. As with the Bardenpho system, it was necessary to add HCl to the aerobic reactors of the UCT system, to maintain pH in the region 7.0 to 7.6; an increase in the acetate fraction of the influent necessitated a corresponding increase in the HCl added. The P removal versus time data, after the acetate concentration was increased from 350 to 500 mgCOD/l, is shown in Fig 6.7. This plot is instructive also in that it showed that stable P removal could be obtained for more than 100 days. The data obtained on P release and uptake (and hence removal) versus acetate added is shown plotted in Fig 6.8(a and b); the responses are near linear. Again, from these responses, it is clear that an enhanced culture of polyP organisms had been attained.

In both the modified Bardenpho and the UCT systems, some of the characteristics of the enhanced cultures were evaluated from the results during the steady state periods. These are listed in Table 6.6 and compared with the same parameters obtained on a UCT system with 20 days sludge age treating unsettled municipal wastewater also of 500 mgCOD/l [see Table 6.3(a) for characteristics of municipal wastewater]. The P/VSS ratios listed in Table 6.6 were obtained from mass balances of the average P removals and sludge wastages, and from averages of the measurement of the volatile solids. These ratios were checked by doing a number of direct measurements of P and volatile solids on the sludges. Statistically, the two values were not significantly different. Clearly, the sludges developed in the enhanced culture systems differed markedly from that found in normal activated sludge systems - the behaviour of the polyP organisms dominated in the enhanced culture systems whereas it was virtually swamped in the activated sludge system receiving

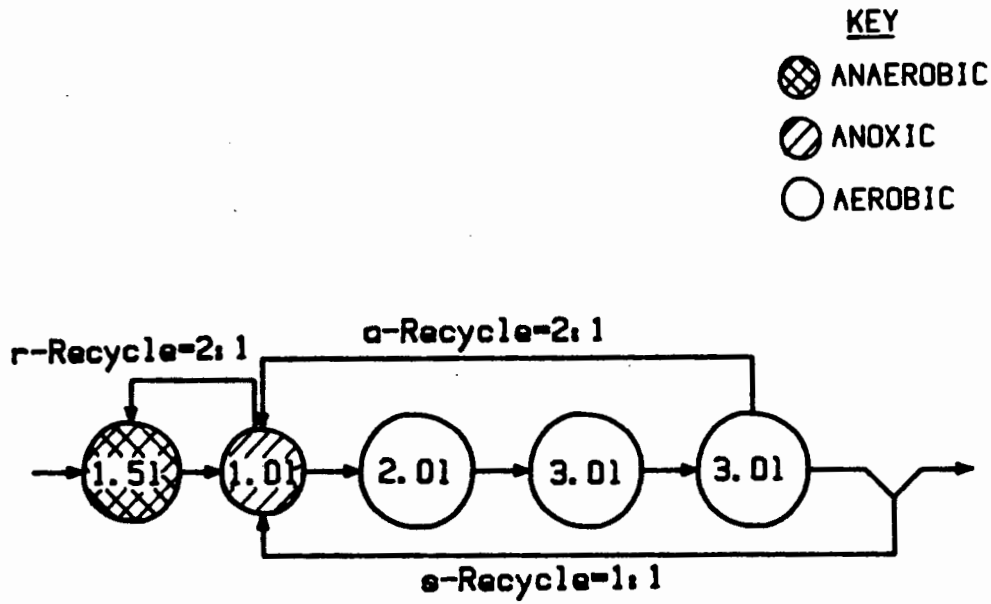


Fig 6.6: Schematic layout of UCT system; reactor volumes in litres, sludge age 10 days and influent flow rate 15 l/d.

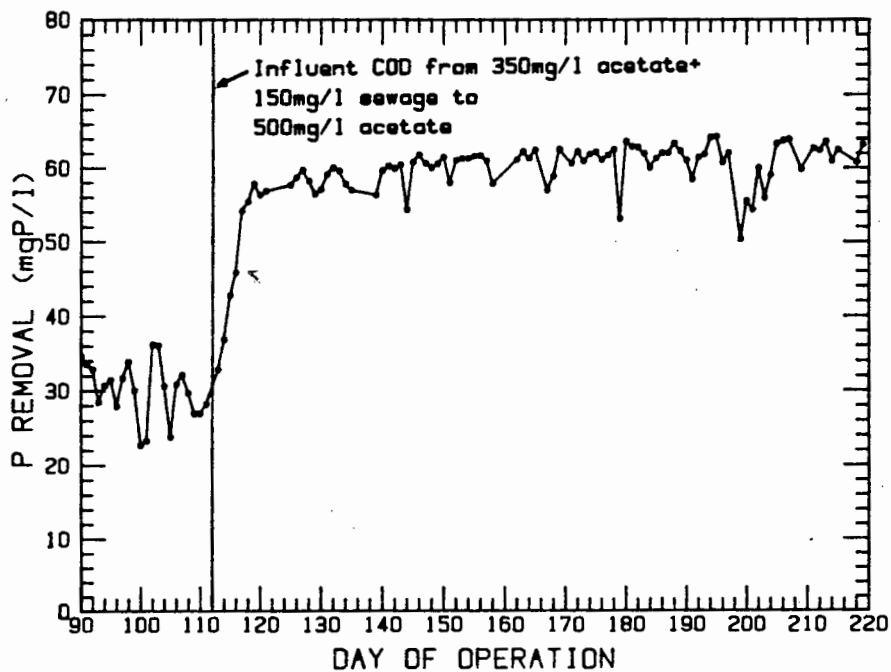


Fig 6.7: Total soluble phosphorus (P) removal versus time in the UCT system (Fig 6.6) when the influent was changed from 350 mgCOD/l acetate + 150 mgCOD/l unsettled municipal sewage to 500 mgCOD/l acetate on day 112.

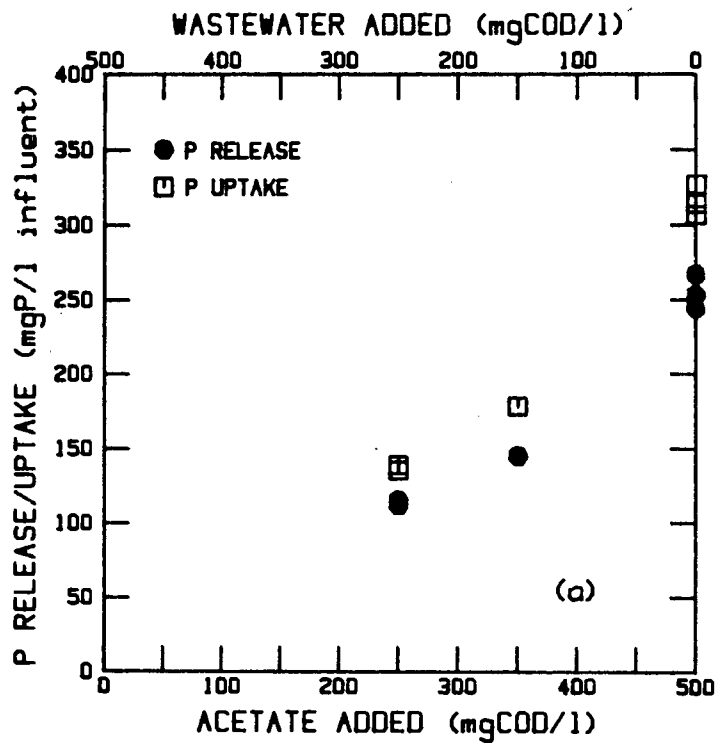


Fig 6.8(a): Total soluble phosphorus (P) release and uptake in UCT system (Fig 6.6) with increasing concentration of acetate in a total influent COD of 500 mg/l (wastewater + acetate).

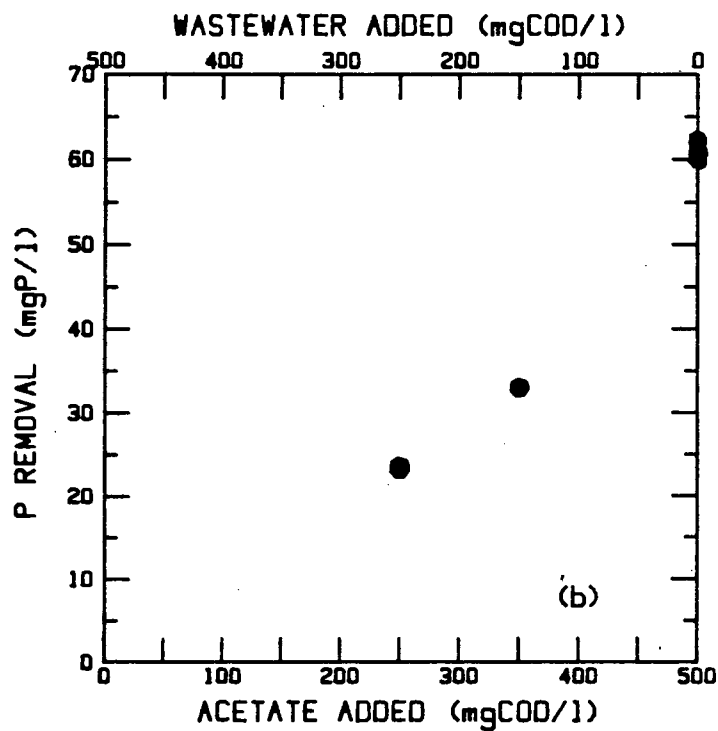


Fig 6.8(b): Total soluble phosphorus (P) removal for data in Fig 6.8(a).

municipal wastewater as influent. This enhanced culture served as the base material for studying the *kinetic behaviour* of the polyP organisms, see Chapter 7.

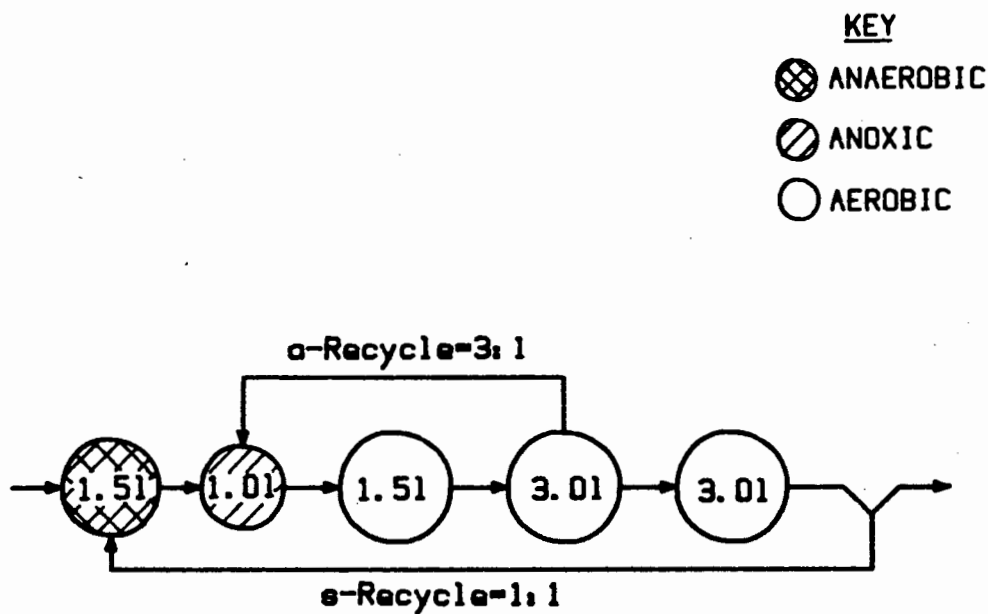
4. SYSTEM BEHAVIOUR AND PROBLEMS

4.1 Decline in P uptake rate

After the main problems regarding nutrients etc. had been resolved, on a number of occasions under steady state operation with an acetate-sewage mixture as influent, a slow decline in P release, uptake and removal was observed. On each occasion deterioration in system response coincided with a new batch of sewage in the sewage-acetate mixture. Initially it was thought that the new batch of sewage was deficient in some essential nutrient. For this reason the influent concentrations of a number of nutrients were sequentially increased over a period of two weeks. Increasing the influent TKN, Mg^{2+} , Ca^{2+} , yeast extract and micro-nutrient concentrations had no effect on P release and removal. However, on changing to another sewage batch, P release, uptake and removal improved. During a period of decline a characteristic response was that P uptake continued to take place in the settling tank - as if the uptake rate had declined in the aerobic reactors. The relative magnitudes of P uptake in the aerobic reactors indicated that the P uptake reaction is of a first order nature, an observation verified in batch tests. It was reasoned, in consequence, that uptake could be improved if the aerobic zone was converted to operate in a more plug flow fashion by recycling sludge from the second aerobic reactor to the anoxic reactor (a-recycle), and not from the last aerobic reactor (see Fig 6.9). Making this change to the modified Bardenpho system indeed did improve the uptake and removal (verified by making a similar change to the parallel UCT system). For the modified Bardenpho system, except for odd batches of sewage, the change caused the response of the system to be more stable. With odd batches of sewage again it was noted that lower removal was associated with P uptake in the settling tank; again it was concluded that the polyP organisms were not being given sufficient time to utilize all the stored PHB - it would appear that if polyP organisms entered the anaerobic zone retaining unused PHB from the preceding aerobic phase, the metabolic controls of the organisms suffered a disturbance. Also, incomplete utilization of PHB resulted in incomplete uptake of P. Thus, for the Bardenpho system, it was decided to aerate the underflow sludge prior to entry into the anaerobic reactor. The underflow rather than the mixed liquor was aerated because its concentration is double that of the mixed liquor (for an underflow recycle ratio of 1:1) and thus a much smaller size reactor is needed to obtain the desired mass fraction. A mass fraction of 9 percent was selected, giving an actual retention

Table 6.6: Characteristics of an enhanced culture and a 'normal' mixed culture UCT system.

Parameter	Enhanced cultures		'Normal' UCT mixed culture
	Bardenpho	UCT	
Sludge age (d)	20	10	20
Influent substrate	acetate	acetate	unsettled municipal wastewater
Influent COD (mgCOD/l)	544	543	500
Effluent COD (mgCOD/l)	62,1	64,9	40
P removal- ΔP (mgP/l)	49,7	60,9	10
$\Delta P/\Delta COD$ (mgP/mgCOD)	0,10	0,12	0,02
P/VSS (mgP/mgVSS)	0,38	0,38	0,15
VSS/TSS (mgVSS/mgTSS)	0,46	0,46	0,75-0,85

**Fig 6.9:**

Schematic layout of 3-stage modified Bardenpho system with a-recycle from second aerobic reactor; reactor volumes in litres, sludge age 20 days and influent flow rate 15l/d.

time of 0,8 hours. The reactor was completely mixed and aerated to maintain oxygen at about 2-4 mgO/l.

Immediately after introducing the aeration reactor into the modified Bardenpho system (receiving an influent mixture of 400 mgCOD/l acetate and 100 mgCOD/l sewage), P removal increased and stabilized, while P release decreased (from 240 to 210 mgP/l influent, i.e. 0,48 to 0,42 mgP/mg influent COD). P uptake now apparently was complete because a net *release* of P of about 15 mgP/l influent was observed in the reaeration reactor. In this instance, provision of this extra aerobic zone very positively lent stability to the system. On introduction of the aeration reactor, there was a change in the P behavioural pattern in the anoxic reactor. Prior to underflow aeration a net P *uptake* was observed in the anoxic reactor; after underflow aeration commenced a net P *release* of about 5-10 mgP/l influent was observed, a very small mass compared to the total release in the anaerobic reactor. However, in a later series of tests when again a polyP organism mass was developed from a normal activated sludge inoculum, insertion of an underflow reaeration reactor did not lead to an improvement. This indicated that there still were other factors that also influenced the stable development of an enhanced culture, see the section following.

4.2 Fluctuating decline in system response

After about six months operation of the systems at steady state with pure acetate, yeast extract and mineral nutrient influent, system P response started to exhibit fluctuations and a gradual deterioration in the P response was observed. Initially it was thought that this deterioration was due to a micro-nutrient deficiency - in Cape Town there are three sources for the water in the distribution system; these differ significantly in chemical quality. It was surmised that some of the source water may be deficient in micro-nutrients, not provided in the added recipe. Consequently, it was decided to add to the influent as complete a micro-nutrient recipe as possible. The most extensive recipe in the literature is that of Kaiser and Hanselmann (1982). This recipe includes the elements Ni and Al in addition to those listed in Table 6.5. Accordingly these elements were added to the influent in the ratios suggested by Kaiser and Hanselmann. This, however, did not improve the response - P removal continued to decline, eventually to 10 mgP/l influent (0,02 mgP/mgCOD), well below the expected 50 mgP/l influent (0,1 mgP/mgCOD). The apparent independence of the system P performance on the influent micro-nutrient recipe led to the conclusion that some change had occurred in the structure of the bacterial population. An analysis of the sludge population using the API method indicated

that only 25 percent of the organisms cultured aerobically were *Acinetobacter* spp; the dominant primary organisms in the system were *Pseudomonas* spp. Although *Pseudomonas* spp. have been shown to accumulate P (Suresh *et al.*, 1984), this accumulation clearly is not of the same magnitude as the P accumulation exhibited by *Acinetobacter* spp.

The reasons for the gradual progress to dominance of the *Pseudomonas* spp. were not clear, but its commencement appeared to be associated with some malfunction of the system (e.g. blocking of tubes causing mixed liquor overflow) and/or its operation (e.g. breakdown of a pump). When, for example, the feed pump broke down, the practice was to feed the remaining daily feed at an augmented rate so as not to disturb the long term steady state. Often, when this was done acetate 'leaked' through the anaerobic reactor; it seems that the leakage initiates and promotes the growth of *Pseudomonas* spp. in the anoxic and aerobic zone, starting the process towards dominance of these organisms. Leakage of acetate also brings about an associated reduction in the growth of polyP organisms, eventually to place the polyP organisms at a competitive disadvantage in the anaerobic/anoxic/aerobic sequencing system. It is essential that this situation is not allowed to occur; great care must be taken not to 'overload' the polyP organisms in the anaerobic reactor to such a degree that leakage of acetate occurs from this reactor. This can be accomplished by observing two operational procedures;

- (1) limiting the acetate load increments at any stage such that no, or very little, acetate is measured in the last anaerobic reactor and/or,
- (2) enlarging the anaerobic reactor sufficiently so that all the acetate is sequestered and any perturbation in acetate load or system operating conditions are accommodated.

Selection of the anaerobic mass fraction is perhaps the most important decision in developing enhanced cultures of polyP organisms. At 10 to 20 day sludge ages, although an anaerobic mass fraction of 8 to 10 percent should be sufficient, when developing the enhanced culture anaerobic mass fractions of more than 20 percent are suggested. (For shorter sludge ages, say 7.5 days, a 50 percent anaerobic mass fraction is required).

To illustrate the instability that can develop in the system with small anaerobic mass fractions, a description of the subsequent development of an enhanced culture is

instructive. A 3-stage modified Bardenpho system at 10 days sludge age with anaerobic mass fraction of 16 percent, see Fig 6.10, was started with mixed liquor from a laboratory scale UCT system receiving unsettled municipal wastewater. Acetate served as the sole influent substrate, with yeast extract and mineral nutrients added according to Table 6.5. The P removal versus time plot is shown in Fig 6.11. The plot shows that when on day 12 the influent acetate concentration was increased from 250 to 350 mgCOD/l, the system did not respond in a satisfactory manner; the removal decreasing to a minimum value by day 32. However, when on day 41 the anaerobic mass fraction was increased from 16 to 32 percent, the P removal increased in a fluctuating manner until the maximum removal was achieved, around day 78. This response highlights the importance of having a sufficiently large anaerobic mass fraction. Also, it is clear from Fig 6.11 that the system may require considerable time to develop a 'mature' enhanced culture from an activated sludge inoculum. If a polyP organism culture inoculum is used, see Fig 6.7, the enhanced culture develops rapidly.

Details of procedures recommended for the development of enhanced cultures of polyP organisms are given in Appendix D.

4.3 Settling behaviour

Of interest is the settling response of the mixed liquor in both the modified Bardenpho and the UCT systems. Settling behaviour tended to follow P removal behaviour in a consistent fashion: A deterioration in P removal through operator error or system malfunctions or acetate overloading always resulted in a deterioration in the settling characteristics, indicated by an observable rise of the sludge level in the settling tank.

When settling was good, operational problems were experienced due to the extremely high settling rate and rapid clumping of the sludge. These caused blocking of the tubes connecting the reactors and formation of sludge bridges in the settling tank over the sludge draw off point through which the liquid readily filtered. To prevent the bridging effect in the sludge, a recycle was introduced from the underflow to the settler influent line and effectively resolved the settler problem.

5. DISCUSSION

From the experimental work outlined above it is evident that:

- (1) An enhanced culture of polyP organisms can be developed successfully by

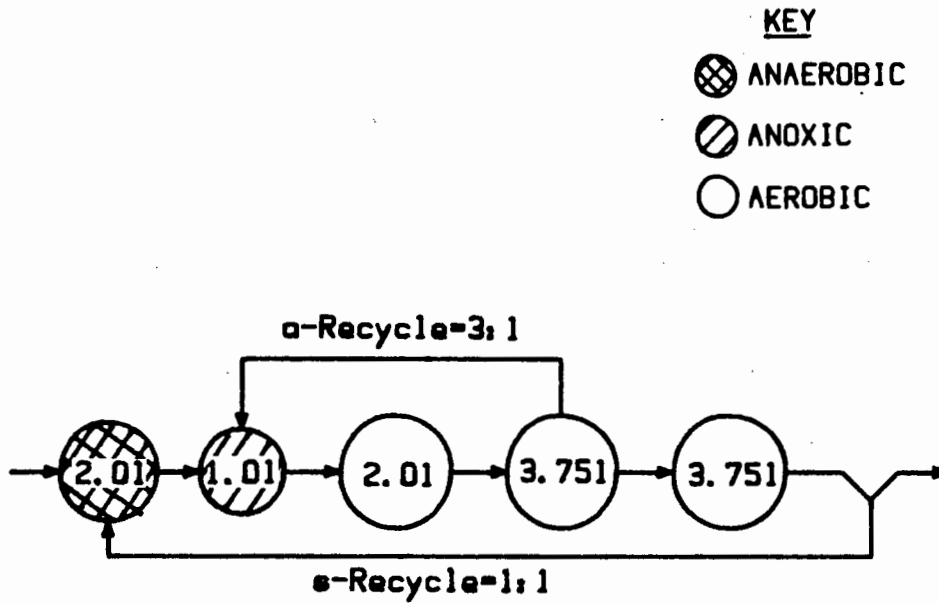


Fig 6.10: Schematic layout of 3-stage modified Bardenpho system; reactor volumes in litres, sludge age 10 days and influent flow rate 15ℓ/d.

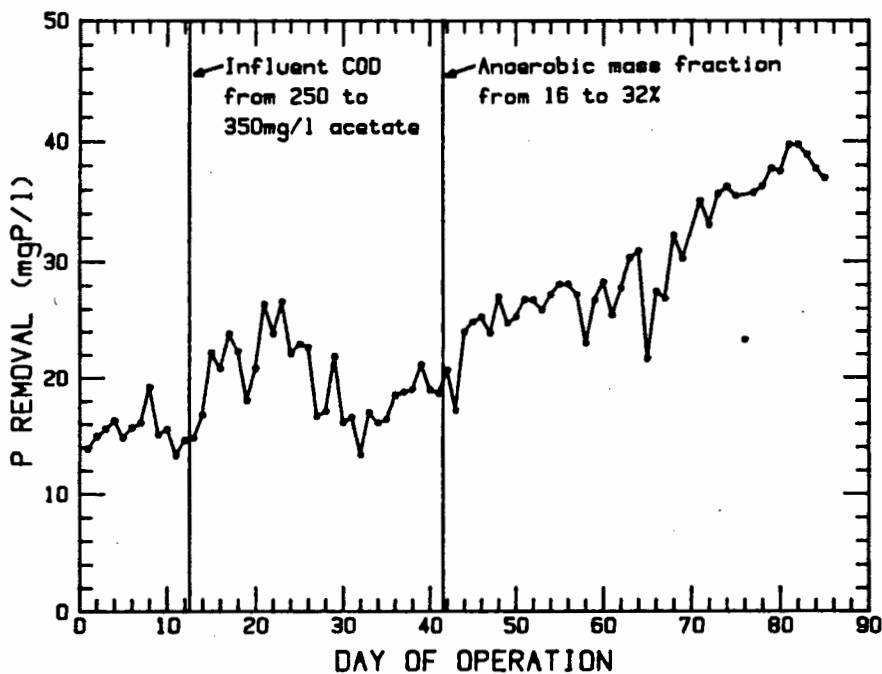


Fig 6.11: Total soluble phosphorus (P) removal versus time in the modified Bardenpho system (Fig 6.10); influent changed from 250 mgCOD/ℓ acetate to 350 mgCOD/ℓ acetate on day 12 and anaerobic mass fraction increased from 16 to 32 percent on day 41.

addition of acetate to the anaerobic reactor of an anaerobic/aerobic sequencing activated sludge system. When acetate served as the sole carbon and energy source to such a system, provided mineral nutrients and yeast extract also were added, almost 100 percent of the organisms cultured aerobically were identified, using the API method, to be *Acinetobacter* spp., a polyP organism. In this enhanced culture the behaviour of the polyP organisms dominated the system response.

- (2) Inorganic nutrients play a vital role in polyP accumulation. Potassium and magnesium are essential in biological excess P removal as these serve as counter-ions for stabilization of the polyP chains. Calcium also has an important function in biological excess P removal, probably as a co-factor in biochemical reactions, and perhaps as another counter-ion in polyP stabilization. These three elements, if present in insufficient quantities, limit excess P removal.
- (3) Yeast extract appears to be essential in developing and operating enhanced cultures of polyP organisms with a single substrate. The yeast extract provides growth factors essential for cell metabolism. In mixed cultures receiving multiple substrates these growth factors probably are supplied by other organism types present in the system.
- (4) The P uptake reaction appears to be first order – improved system operation is attained by operating aerobic reactors as plug flow rather than as a completely mixed single reactor. There is tentative evidence that if the utilization of PHB is not complete in the aerobic reactors it may adversely affect the system P response. To overcome this problem it may prove beneficial to include an aeration reactor in the underflow recycle stream.
- (5) The biological P release has a minor effect on anaerobic reactor pH, provided pH is close to 7.0. However, P uptake increases the pH of the mixed liquor in its passage through the series system of aerobic reactors. In enhanced culture systems, aerobic pH has been observed to increase to above 9, which can cause collapse of the system. With enhanced culture systems it is necessary always to add acidity to each of the aerobic reactors to maintain the pH near about 7.5. A detailed biochemical explanation of this phenomenon has been given in Chapter 5.

- (6) In anaerobic/aerobic systems with acetate as substrate, *Pseudomonas* spp. may replace *Acinetobacter* spp. as the dominant primary organisms. All the factors giving rise to this shift in bacterial population structure are not yet clear, but certainly overloading of the organism mass in the anaerobic reactor with acetate can induce this shift by 'leakage' of acetate through the anaerobic reactor to the aerobic zone. This can be countered by (i) providing an adequately large anaerobic mass fraction, (ii) limiting the acetate load increments and (iii) minimizing acetate load perturbations.

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CHAPTER 7

ENHANCED POLYP ORGANISM CULTURES – EXPERIMENTAL BEHAVIOUR

1. INTRODUCTION

In Chapter 6, experimental procedures were reported whereby enhanced cultures of polyphosphate (polyP) organisms were developed in the modified Bardenpho and UCT systems. The mixed liquors in these systems were shown to comprise *Acinetobacter* spp., a polyP organism, in excess of 90 percent. It was envisaged that, in these enhanced culture systems, the behaviour of the polyP organisms could be readily isolated because of the relatively pure assembly of these organisms. Furthermore, by developing the enhanced cultures in modified Bardenpho and UCT systems and by not positively excluding other organisms (e.g. predators), the polyP organism behaviour could be expected to conform reasonably closely to their behaviour in the normal mixed culture systems.

This chapter reports on experimental investigations, using the enhanced cultures to:

- (1) identify the compounds influenced by biological excess phosphorus (P) removal,
- (2) identify the processes that act on these compounds and,
- (3) conceptualize a mechanistic model that qualitatively describes the kinetic and stoichiometric behaviour of the processes and compounds.

2. BACKGROUND

To set up an experimental protocol to achieve the objectives outlined above, one needs to have some initial conceptualizations of the processes and compounds that need to be monitored in the experiments, and the conditions under which these processes can be isolated and stimulated, i.e. some rudimentary conceptual model. As more information becomes available from the experimentation, aspects of the rudimentary model can be verified or improved on. This in turn provides guidance on further experimental tasks that need to be undertaken, so that both the model and the experimental investigation tend to evolve contemporarily. This certainly was the path followed in this study. Initial conceptualization of the rudimentary model, and the experiment techniques used to obtain information were strongly

influenced by:

- (1) The approaches developed by Marais and Ekama (1976), Dold *et al.* (1980) and van Haandel *et al.* (1981) in setting up a model to describe general activated sludge kinetics under aerobic, anoxic and anaerobic conditions.
- (2) The biochemical model describing biological excess P removal, reported in Chapter 5.

Marais and co-workers found that description of the behaviour of activated sludge systems must include the growth (substrate utilization) and endogenous mass loss processes. In the stoichiometry of growth, for every unit of substrate consumed, it is accepted that a constant fraction (the true specific yield) appears as new cell mass and the remainder is oxidized to generate energy for synthesis giving rise to an associated oxygen requirement. When attempting to obtain an estimate of the true specific yield, Marais and co-workers found that it cannot be determined directly from sludge production in systems operated under steady state because the active mass synthesized is subject to endogenous/maintenance/death processes. These processes not only reduce the active mass but also generate inert (endogenous) residues, both particulate and soluble, so that the observed volatile solids not only are less than that indicated by the true specific yield, but also are made up of active and endogenous particulate fractions, to give an apparent yield. Due to these endogenous effects, the apparent yield decreases as the sludge age of the system increases. If it is accepted that the endogenous residue generation is constant with respect to the active mass that disappears, then it is possible to obtain estimates of the true specific yield and endogenous mass loss by trial and error curve fitting to observed yields in a set of steady state responses over a range of sludge ages. However, Marais and Ekama (1976) found that the yield and endogenous mass loss effect act in a compensatory fashion; good 'fits' can be obtained by different pairs of true specific yield values and endogenous mass loss rates. They found it essential to obtain an independent assessment of one of the two processes. To achieve this, they established that the endogenous mass loss rate could be isolated by doing aerobic digestion batch tests on samples of mixed liquor without addition of substrate. From a semilog plot of the oxygen utilization rate versus time, they determined that the endogenous mass loss process conformed to first order kinetics with respect to the active mass and that the slope of the plot defines the specific endogenous mass loss rate. Applying this rate to the steady state systems over a range of sludge ages, and accepting the value for the unbiodegradable particulate endogenous residue fraction

of the active mass measured by McCarty and Brodersen (1962) and Washington and Hetling (1965), they determined the true specific yield. In the enhanced cultures, in order to obtain information on the yield and endogenous mass loss, it was proposed to follow the approach of Marais and co-workers by:

- (1) Running two steady state polyP organism enhanced culture systems at 10 and 20 days sludge ages respectively at 20° C, to obtain apparent yield values.
- (2) Conducting aerobic digestion batch tests on mixed liquor samples drawn from the respective steady state systems in order to determine endogenous mass loss characteristics, including stoichiometry and kinetics of the mass loss, generation of endogenous residue fractions and changes in stored polyP.

The biochemical model in Chapter 5 describes the behavioural patterns of the biochemical processes associated with biological excess P removal. From the biochemical model it is apparent that the approach of Marais and co-workers is not sufficient; due account must be taken of a number of additional processes and compounds that are unique to biological excess P removal. The biochemical model provides guidance on the selection of the processes and compounds and the essential requirements that make the excess P removal system work, namely an anaerobic/aerobic sequence with short chain fatty acids present in the anaerobic stage. Also, the biochemical model can be used to develop experimental procedures because it identifies the conditions under which the short chain fatty acids are sequestered by the polyP organisms, with associated P release and, the conditions for subsequent utilization of the sequestered acids for growth with associated P uptake and polyP synthesis. To obtain information on the relevant processes and compounds, including kinetic information, it was proposed that a number of batch tests be undertaken (at 20° C) using mixed liquor from the steady state enhanced culture systems:

- (1) Add acetate under anaerobic conditions to observe sequestration of the acetate and associated P release.
- (2) Repeat (1) above but follow with aeration to observe P uptake and oxygen utilization rates, the latter giving a measure of stored substrate utilization for synthesis of new cell mass and for P uptake.

- (3) Repeat (2) above but, instead of aerating, add nitrate to the batch to observe denitrification behaviour.
- (4) Add acetate under *aerobic* conditions to observe aerobic acetate sequestration.

3. STEADY STATE RESPONSE PATTERN

3.1 First test series

The two system configurations in which the enhanced cultures were developed are shown in Fig 7.1(a and b). Essentially the two systems conform to a 3-stage modified Bardenpho and a UCT system respectively. The enhanced culture systems were operated according to the procedures described in Chapter 6 and Appendix D. The modified Bardenpho system was operated at 20 days sludge age and the UCT system at 10 days. Sludge age was maintained by hydraulic control, i.e. drawing off the required waste mixed liquor volume from the last aerobic reactor, see Marais and Ekama (1976). The substrate was sodium acetate, 500 mgCOD/ ℓ ; macro-nutrients N (35 mgN/ ℓ), P (54-65 mgP/ ℓ), Mg (30 mgMg/ ℓ), K (149 mgK/ ℓ) and Ca (15 mgCa/ ℓ); micro-nutrients and growth factors as listed in Table 7.1. In both systems a sidestream of acid (hydrochloric) was added to each of the aerobic reactors to maintain the pH in the region $\pm 7,5$; failure to do so resulted in a sharp increase in the aerobic zone pH to values greater than 9 (as against $\pm 7,1$ in the anaerobic zone). The necessity for acid addition is in accordance with the biochemical model (Chapter 5) which predicts that the pH in the anaerobic zone would not change significantly, but would rise in the aerobic zone.

After a system had attained steady state, the system response was monitored for an extended period, about four months. The following parameters were measured; unfiltered influent and filtered effluent COD, TKN, total P and nitrate (NO_3); filtered reactor contents total P and NO_3 ; pH in all reactors; oxygen utilization rates (OUR) in the aerobic reactors; total suspended solids (TSS) and volatile suspended solids (VSS) of the waste mixed liquor. Population structures of the mixed liquor samples also were analyzed by the Scientific Services branch of the Johannesburg City Council using the Analytical Profile Index (API) procedure.

The averages of the results of all tests over the steady state periods are summarized in Table 7.2. Mass balances on the COD and nitrogen give recoveries of 92 and 103 percent respectively for the modified Bardenpho system and 90 and 104 percent respectively for the UCT system. Recoveries of greater than 90 percent usually are

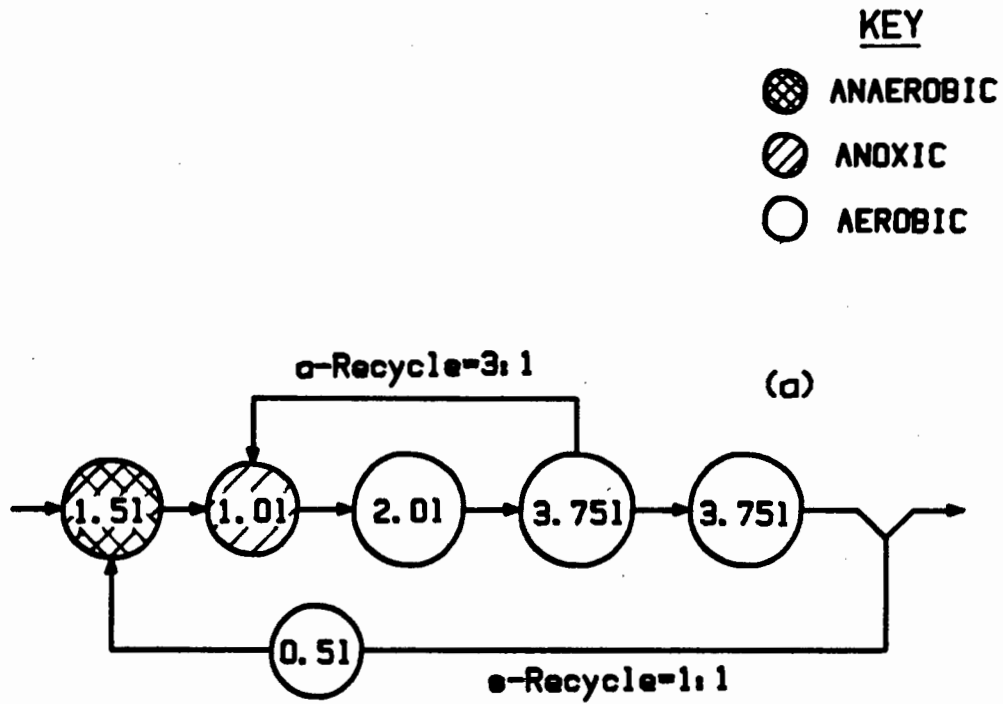


Fig 7.1(a): Schematic layout of 3-stage modified Bardenpho polyP organism enhanced culture system.

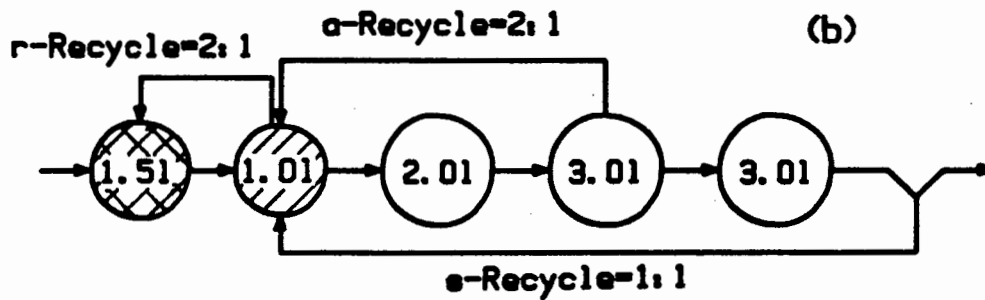


Fig 7.1(b): Schematic layout of UCT polyP organism enhanced culture system.

Table 7.1: Influent micro-nutrients added per 1000 mgCOD sodium acetate.

Chemical	mg added/1000 mgCOD sodium acetate	
	Compound	Element
Yeast extract	50	
FeSO ₄ ·7H ₂ O	5,25	1,05 Fe
ZnSO ₄ ·7H ₂ O	1,5	0,34 Zn
MnSO ₄	1,5	0,55 Mn
CuSO ₄ ·5H ₂ O	0,3	0,076 Cu
CoCl ₂ ·6H ₂ O	0,3	0,074 Co
Na ₂ MoO ₄ ·2H ₂ O	0,15	0,059 Mo
H ₃ BO ₃	0,3	0,052 B
KI	0,075	0,057 I

Table 7.2: Steady state response of the 20 day sludge age 3-stage modified Bardenpho and 10 day sludge age UCT enhanced culture systems.

3 STAGE BARDENPHO SYSTEM								
Parameter	Value	Parameter					Value	
Substrate	Acetate	VSS/TSS (mgVSS/mgTSS)					0,48	
Sludge age (d)	20	ΔVSS/influent COD (mgVSS/mgCOD)					0,24	
Flow rate (l/d)	15	P removal (mgP/l)					49,7	
% <i>Acinetobacter</i> spp.*	90	P/VSS (mgP/mgVSS)					0,38	
VSS (mgVSS/l)	2998	ΔP/influent COD (mgP/mgCOD)					0,09	
TSS (mgTSS/l)	6181							
	Influent	Reactor					Effluent	Underflow aeration
		1	2	3	4	5		
COD (mgCOD/l)	544	-	-	-	-	-	62	-
TKN (mgN/l)	35,2	-	-	-	-	-	2,7	-
PO ₄ (mgP/l)	53,6	145,8	76,8	49,1	23,4	5,6	3,9	22,2
NO ₃ (mgN/l)	0	1,0	7,4	11,4	12,0	11,5	11,5	10,3
OUR (mgO/l/hr)	-	-	-	38,5	17,8	9,5	-	14,0
PO ₄ change (mgP/l influent)**	-	+215,7	+22,4	-138,6	-128,6	-39,1	-	+18,4
NO ₃ change (mgN/l influent)**	-	-8,2	-1,3	+20,0	+3,3	-1,1	-	-1,2
pH	-	7,19	7,33	7,47	7,59	7,65	-	7,56
U C T SYSTEM								
Parameter	Value	Parameter					Value	
Substrate	acetate	VSS/TSS (mgVSS/mgTSS)					0,46	
Sludge age (d)	10	ΔVSS/influent COD (mgVSS/mgCOD)					0,30	
Flow rate (l/d)	15	P removal (mgP/l)					60,9	
% <i>Acinetobacter</i> spp.	90	P/VSS (mgP/mgVSS)					0,38	
VSS (mgVSS/l)	2397	ΔP/influent COD (mgP/mgCOD)					0,11	
TSS (mgTSS/l)	5224							
	Influent	Reactor					Effluent	
		1	2	3	4	5		
COD (mgCOD/l)	543	-	-	-	-	-	65	
TKN (mgN/l)	36,0	-	-	-	-	-	2,7	
PO ₄ (mgP/l)	63,7	157,5	86,3	48,2	12,7	4,8	2,9	
NO ₃ (mgN/l)	0	0,5	2,9	7,1	8,0	7,0	7,0	
OUR (mgO/l/hr)	-	-	-	39,5	17,5	9,0	-	
PO ₄ change (mgP/l influent)**	-	+236,2	+17,0	-152,6	-141,7	-19,7	-	
NO ₃ change (mgN/l influent)**	-	-4,4	-7,0	+16,9	+3,6	-2,0	-	
pH	-	7,18	7,27	7,30	7,59	7,61	-	

* % of organisms cultured aerobically determined to be *Acinetobacter* spp. using API.

** + indicates P release/nitrification, - indicates P uptake/denitrification.

indicative of acceptable mass balances, Marais and Ekama (1976). Mass balances on P and nitrate were calculated relative to the influent flow and are shown in Table 7.2. From the mass of P removed each day and the mass of volatile solids wasted per day, the P/VSS ratio was calculated. This ratio was checked by doing a number of direct measurements of P and volatile solids on the mixed liquor. Statistically, the two values were not significantly different. The ratios also are given in Table 7.2.

From Table 7.2, taking the UCT system as an example, the most striking features in the system response are:

- (1) The specificity of the population structure; more than 90 percent of the organisms cultured aerobically were identified to be *Acinetobacter* spp. using the API procedure.
- (2) The extremely high phosphorus content of the sludge in the aerobic reactor, 0,38 mgP/mgVSS. This gives rise to a VSS/TSS ratio of 0,46 mgVSS/mgTSS, as against the usual 0,75-0,85 in activated sludge systems.
- (3) The magnitudes of the P release, uptake and removals; 253 mgP/l release (anaerobic + anoxic reactors), 314 mgP/l uptake (aerobic reactor) giving a net removal of 61 mgP/l (concentrations with respect to influent flow).
- (4) The high filtered effluent COD concentration. All the substrate acetate was removed in the anaerobic and anoxic zones and an apparently unbiodegradable COD was generated in the system, giving a net effluent COD concentration of 65 mgCOD/l. This effluent value is considerably higher than that expected for an acetate feed to a normal activated sludge system, 15-20 mgCOD/l. The most likely reason is the high specificity of the organism assembly - byproducts generated cannot be used by the highly specific organism mass composition of the enhanced culture, whereas in a mixed culture these byproducts are likely to serve as a substrate source for other species.
- (5) The low nitrate (NO_3) removal, about 11 mgN/l. Despite the fact that the influent was nearly totally readily biodegradable, and high concentrations of internally stored acetate (as PHB) were present in the anoxic zone, NO_3 removal was poor indicating that only a small fraction of the *Acineto*-

bacter spp. could use NO_3 as an electron acceptor. This is supported by the observation that usually no P was taken up in the anoxic reactors of the enhanced culture systems. Batch tests with nitrate as electron acceptor verified this behaviour, see later.

- (6) Utilization of NO_3 as a nitrogen source for growth. Nitrification was virtually complete in the first aerobic reactor; in the third aerobic reactor the NO_3 concentration decreased, indicating a utilization of NO_3 as a nitrogen source for synthesis; this was confirmed by batch tests, see later.

3.2 Second test series

The first test series was conducted at sludge ages of 20 and 10 days using the 3-stage modified Bardenpho and UCT systems respectively. A second series of tests then were inaugurated with the objectives to investigate the effects of sludge age and influent COD on the enhanced culture steady state response pattern. Both the systems in the first series gave results consistent with each other. Hence, for reasons of convenience, the system selected for the second series was the 3-stage modified Bardenpho. In the first test series the modified Bardenpho system was operated only at 20 days sludge age; in the second series the system was operated at 10 and 7.5 days sludge age. The influent COD was reduced from the 500 mgCOD/l acetate in the first series to 350 mgCOD/l acetate in the second series; the added mineral nutrients were decreased proportionally. From the developmental work on enhanced cultures in Chapter 6 it was apparent that the anaerobic mass fraction of the 20 day sludge age 3-stage modified Bardenpho system (12 percent) would be too small to enable all the acetate to be sequestered at shorter sludge ages and accordingly the anaerobic mass fraction was increased to 31 percent. This mass fraction was in excess of that theoretically required for complete sequestration but experience had indicated that a larger anaerobic mass fraction than the theoretical was necessary to ensure that no acetate 'leaked' through the anaerobic zone in event of operator error or system malfunction (Chapter 6). The anaerobic mass fraction was divided between two reactors in series. This had the advantage that it allowed the rate of acetate uptake to be determined experimentally in the first reactor. The layout of the system is shown in Fig 7.1(c).

The systems were operated at steady state for 3 to 4 sludge ages using the same operational and monitoring procedures as in the first series.

The average results over the steady state periods are summarized in Table 7.3. Mass balances on the COD and nitrogen give recoveries of 91 and 90 percent respectively for the 10 day sludge age system, and 93 and 95 percent respectively for the 7,5 day sludge age system; these percentage recoveries, as mentioned earlier, indicate that the results are acceptable. Mass balances on P and nitrate were calculated relative to the influent flow, see Table 7.3. Mass balances on the P removed by the systems, and on the mixed liquor wasted per day, were used to determine the P/VSS ratios. These ratios also are given in Table 7.3.

The results obtained in the second series are consistent with those of the first series, viz. low VSS/TSS and P/VSS ratios, large magnitudes of the P release, uptake and removal, high effluent COD's, poor denitrification and utilization of nitrate as a nitrogen source for synthesis.

4. BATCH TESTS

To obtain further information on the different polyP organism processes, e.g. acetate uptake, P release and uptake, growth rates, endogenous mass loss, etc. series of batch tests were undertaken using mixed liquor from the steady state enhanced culture systems. As discussed earlier, five types of batch test were undertaken; 4.1 aerobic digestion of the mixed liquor, 4.2 response with addition of acetate under anaerobic conditions, with either 4.3 aerobic or 4.4 anoxic response thereafter, and 4.5 response with acetate addition under aerobic conditions. Data was accumulated from these batch tests on 4.6 cation release and uptake.

4.1 Aerobic digestion

The objective of this series of batch tests was to obtain information on endogenous mass loss behaviour. The procedure followed for these tests was that developed by Marais and Ekama (1976): Mixed liquor was drawn from the last aerobic reactor of the enhanced culture systems and placed in a continuously stirred batch reactor, without addition of substrate. At the start of the test mixed liquor samples were drawn from the batch for VSS and TSS determinations. The dissolved oxygen (DO) in the batch was monitored continuously by means of a Yellow Springs oxygen meter connected to a strip recorder. An electronic controller turned on aeration when the DO dropped below 5 mgO/L, and turned off aeration when it rose above 7,5 mgO/L. Floating plastic balls were used to cover the surface of the mixed liquor to minimize DO transfer at the air/liquid interface. The rate of DO decrease during the air off period gave the oxygen utilization rate (OUR). The pH in the batch also was monitored continually and controlled to remain in the region 7,4–7,6 by addition of

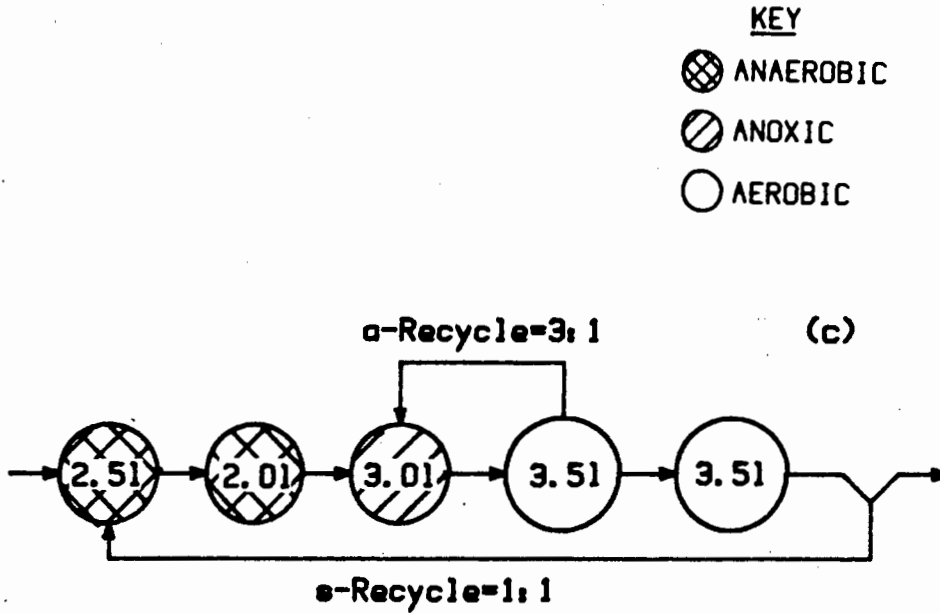


Fig 7.1(c): Schematic layout of 3-stage modified Bardenpho enhanced culture system.

Table 7.3: Steady state response of the 10 and 7,5 day sludge age 3-stage modified Bardenpho enhanced culture systems.

3 STAGE BARDENPHO SYSTEM							
Parameter	Value	Parameter					Value
Substrate	Acetate	VSS/TSS (mgVSS/mgTSS)					0,46
Sludge age (d)	10	Δ VSS/influent COD (mgVSS/mgCOD)					0,27
Flow rate (l/d)	15	P removal (mgP/l)					38,6
VSS (mgVSS/l)	1167	P/VSS (mgP/mgVSS)					0,34
TSS (mgTSS/l)	2547	Δ P/influent COD (mgP/mgCOD)					0,09
Influent		Reactor					Effluent
		1	2	3	4	5	
COD (mgCOD/l)	417	-	-	-	-	-	62
TKN (mgN/l)	26,9	-	-	-	-	-	2,5
PO ₄ (mgP/l)	43,8	115,8	134,0	72,9	38,6	7,6	5,2
NO ₃ (mgN/l)	0	0,3	0,5	2,4	4,0	3,7	3,7
OUR (mgO/l/hr)	-	-	-	-	23,0	8,9	-
PO ₄ change (mgP/l influent)**	-	+181,0	+37,9	-18,9	-172	-66,5	-
NO ₃ change (mgN/l influent)**	-	-3,2	+0,6	-1,06	+8,0	-0,66	-
pH	-	7,09	7,10	7,40	7,71	7,70	-
Parameter		Value	Parameter				
Substrate	acetate		VSS/TSS (mgVSS/mgTSS)				
Sludge age (d)	7,5		Δ VSS/influent COD (mgVSS/mgCOD)				
Flow rate (l/d)	15		P removal (mgP/l)				
VSS (mgVSS/l)	1036		P/VSS (mgP/mgVSS)				
TSS (mgTSS/l)	2283		Δ P/influent COD (mgP/mgCOD)				
Influent			Reactor				Effluent
			1	2	3	4	5
COD (mgCOD/l)	410	-	-	-	-	-	53
TKN (mgN/l)	26,3	-	-	-	-	-	2,5
PO ₄ (mgP/l)	46,0	102,1	132,1	72,6	38,2	8,2	3,4
NO ₃ (mgN/l)	0	0,3	0,6	2,2	3,5	3,3	3,3
OUR (mgO/l/hr)	-	-	-	-	20,4	7,6	-
PO ₄ change (mgP/l influent)**	-	+154,9	+60,0	-15,8	-172,2	-69,6	-
NO ₃ change (mgN/l influent)**	-	-2,7	+0,5	-0,6	+6,4	-0,3	-
pH	-	7,18	7,18	7,45	7,74	7,74	-

** % of organisms cultured aerobically determined to be *Acinetobacter* spp. using API.

** + indicates P release/nitrification, - indicates P uptake/denitrification.

hydrochloric acid or sodium bicarbonate when necessary. At regular intervals, mixed liquor samples were drawn from the batch reactor and filtered immediately. The filtrate was analyzed for total P and NO_3 concentrations.

A number of batch tests were run for periods of up to 10 days; it was found that after about 10 days a qualitative change occurred in the endogenous mass loss behaviour in the batch tests, manifested by an increase in the OUR. Most probably, this was due to the development of predatory and other primary organisms in the batch. Typical response time relationships obtained are shown in Fig 7.2(a and b). The following features merit comment:

- (1) The OUR in Fig 7.2(a) is shown plotted log OUR versus time in Fig 7.3. During the initial stages stored PHB still is available giving rise to a high OUR, but reduces thereafter to that associated with endogenous mass loss only. In the true endogenous phase (> 2 days) the OUR follows a first order rate with respect to the active VSS concentration. The slope of the log OUR defines the specific endogenous mass loss rate constant (b_G), i.e. $b_G = 2,303 \cdot \text{slope}(\log \text{OUR})$. (This slope is independent of nitrification, Marais and Ekama, 1976). The b_G values from a number of such tests are shown plotted on normal probability paper in Fig 7.4 giving a mean value for b_G of 0,04/d and standard deviation of the mean of $\pm 0,004$. This mean value is very low compared with 0,24/d found for normal activated sludge (Marais and Ekama, 1976). This would indicate that very little or no species interaction, such as predation, is present; with 'normal' activated sludge, species interaction has been implicated as the principal cause for the high "endogenous" rate constant observed, Dold *et al.* (1980).
- (2) P and nitrate concentration, Fig 7.2(b), both show an initial decrease, associated with stored PHB utilization, followed by an increase, associated with endogenous mass loss. If the P release is related directly to the volatile mass loss (i.e. lysis of the P content of the mass lost) then the P concentration changes will be stoichiometrically related to the volatile mass loss, so also the nitrate (NO_3). To check this, plots were made of the concentrations of P and NO_3 versus the cumulative oxygen utilized in the digestion tests (i.e. the integral under the OUR curve), in Fig 7.5. Both plots appear to be linear in the endogenous region, thereby indicating that the P released and NO_3 generated are stoichiometrically related to the endogenous mass loss. This conclusion is true irrespective of whether all the

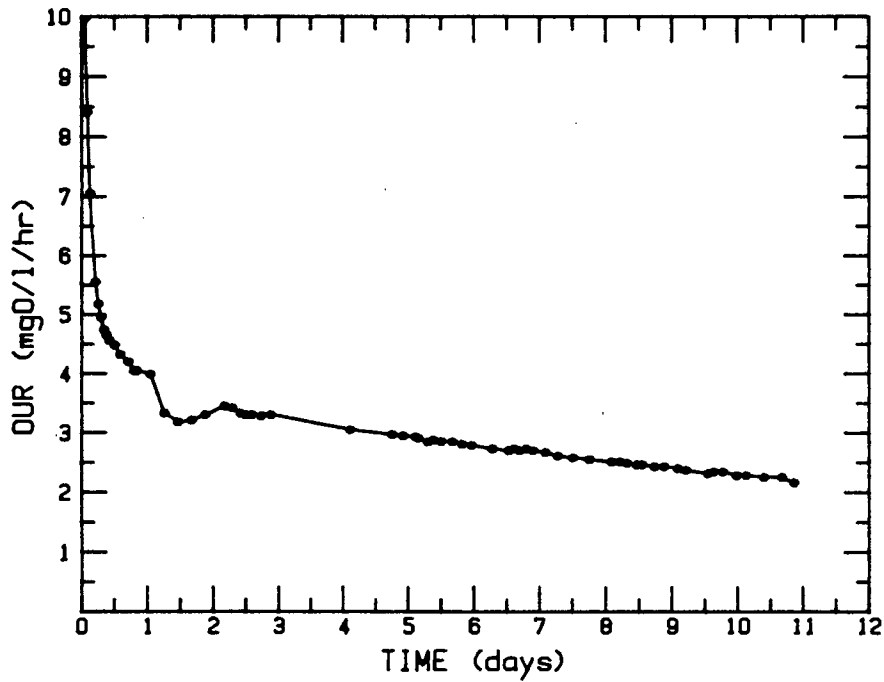


Fig 7.2(a): Oxygen utilization rate (OUR) response with time in a batch aerobic digestion of mixed liquor drawn from the enhanced culture system ($\text{VSS} = 2400 \text{ mgVSS/l}$).

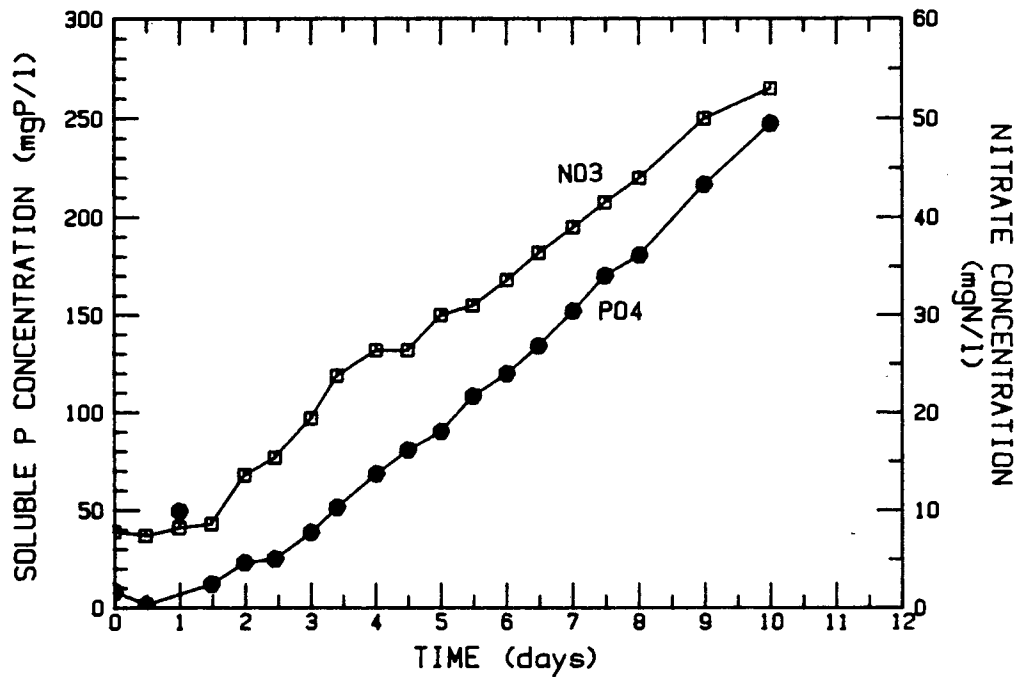


Fig 7.2(b): Total soluble phosphate (PO_4) and nitrate (NO_3) concentration-time profiles for the batch aerobic digestion in Fig 7.2(a).

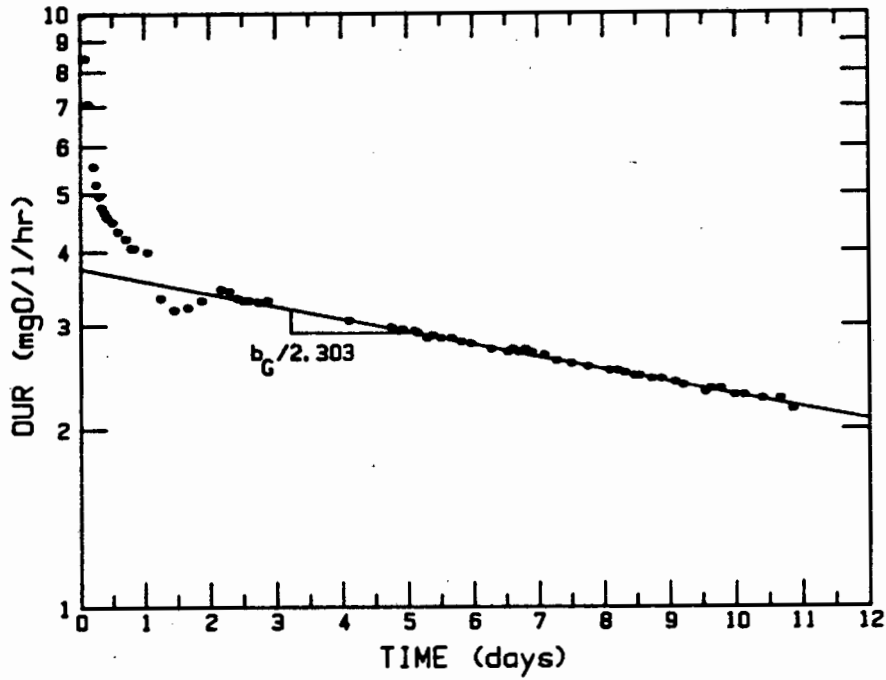


Fig 7.3: Semilog plot of oxygen utilization rate (OUR) versus time for the data in Fig 7.2(a).

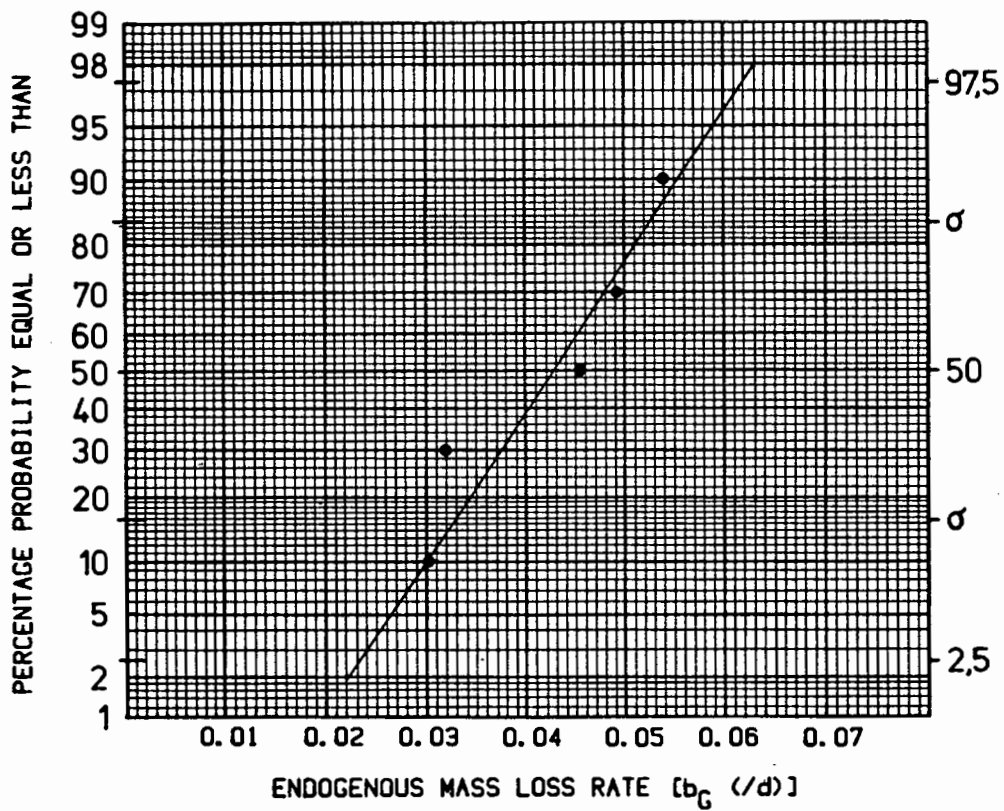


Fig 7.4: Statistical plot of endogenous mass loss rates (b_G) obtained from a number of aerobic digestion batch tests.

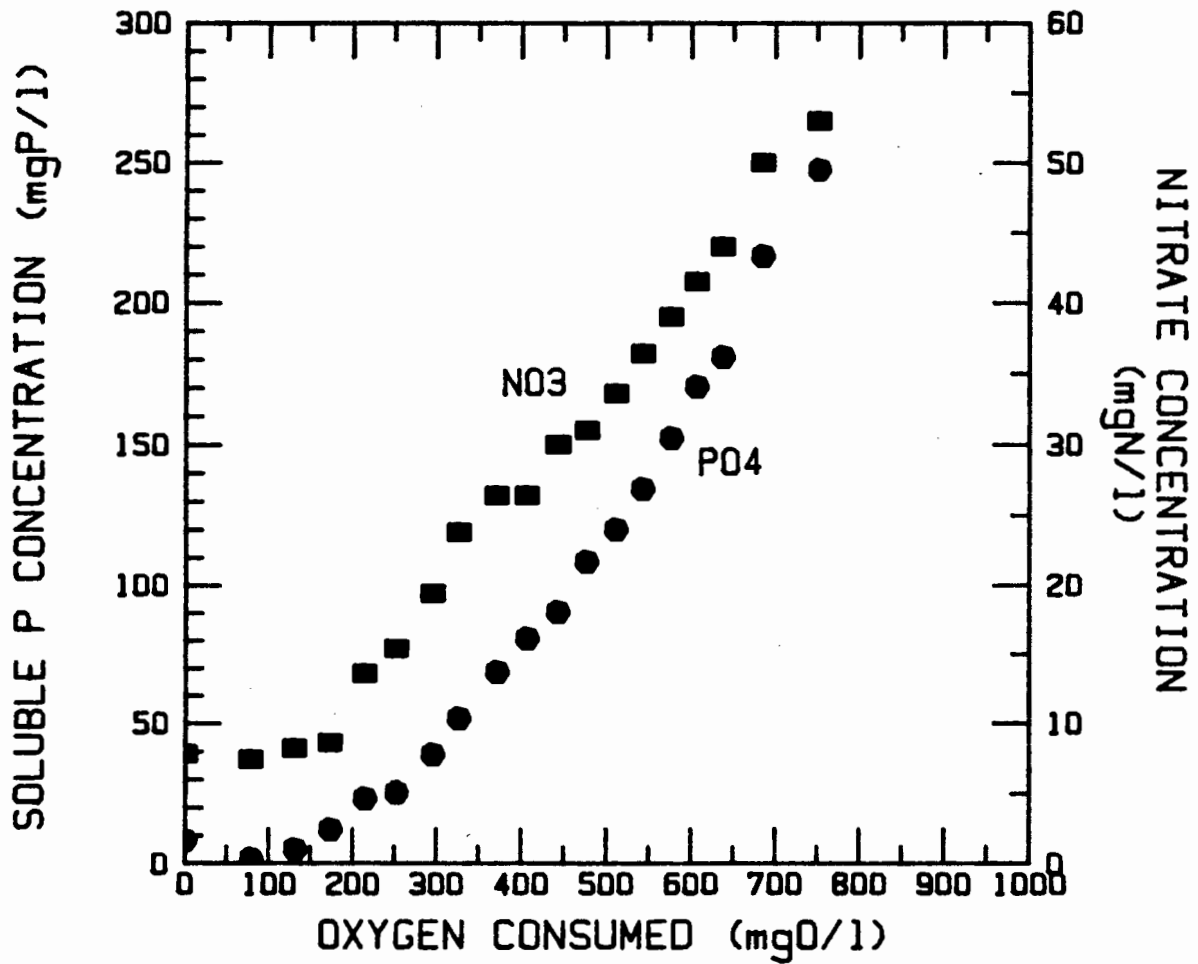


Fig 7.5: Plot of total soluble phosphate (PO_4) and nitrate (NO_3) concentrations [from Fig 7.2(b)] versus cumulative oxygen consumed [from Fig 7.2(a)], in the batch aerobic digestion in Fig 7.2(a and b).

volatile mass lost is oxidized, i.e. endogenous residue is zero, or only a fraction is oxidized with a remaining fraction being unbiodegradable, provided the fraction remains constant with time. Similarly b_G also is independent of the fraction of the endogenous mass loss that is oxidized for energy, again provided the fraction remains constant. Thus, b_G can be obtained directly from experimental observation, but the *fraction* of the endogenous mass loss that is oxidized for energy must be determined by curve fitting the theoretical OUR against the OUR observed in the digestion test, taking due account of nitrification.

Subsequently, when the kinetic model was developed and attempts were made to fit the predicted behaviour in the batch to that observed, it was found necessary to include the generation of a soluble unbiodegradable COD during the endogenous process to obtain consistency between predicted and observed data. To check whether such generation of COD did in fact occur, the aerobic digestion batch tests described above were repeated, monitoring the filtered COD over the test period. Typical results from such a test are shown plotted in Fig 7.6(a, b and c). Referring to Fig 7.6(a and b) the OUR, P and NO_3 concentration profiles conform to those in Fig 7.2 (a and b). With regard to the filtered COD concentration profile [Fig 7.6(c)], the COD increases with time during the course of the batch test. This verifies that, in addition to the usual generation of a particulate endogenous residue during endogenous mass loss, there is a generation of soluble endogenous 'residue' (COD). Most likely soluble residue generation is due to the high specificity of the organism population – byproducts generated during endogenous mass loss processes cannot be utilized by the specific organism mass of the enhanced culture; in normal mixed culture systems, these byproducts very likely would serve as a substrate for other species. (This soluble unbiodegradable COD generation had already been noted earlier in the steady state systems).

4.2 Anaerobic response with acetate addition

The objective of this series of batch tests was to gain information on the behaviour of the anaerobic sequestration reactions with associated P release.

Batches of mixed liquor were drawn from the last aerobic reactor of the enhanced culture systems, diluted appropriately, so as to reduce the rates of reaction per litre batch volume for accurate measurements, and placed in covered, continuously stirred, batch reactors. Nitrogen gas was bubbled continuously through the batch mixed liquor to strip out any oxygen and to prevent oxygen ingress into the batch

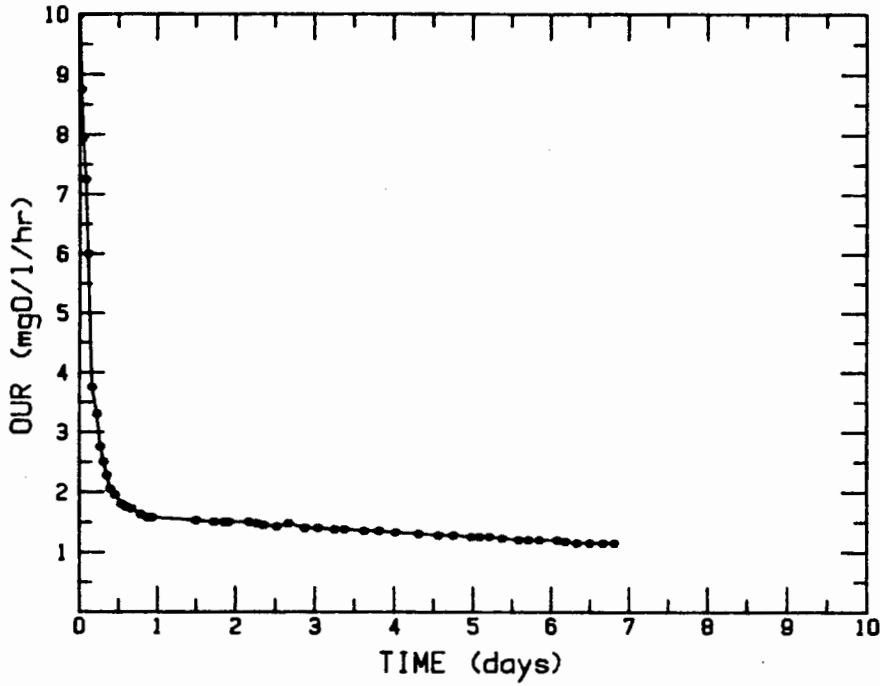


Fig 7.6(a): Oxygen utilization rate (OUR) response with time in a batch aerobic digestion of mixed liquor drawn from the enhanced culture system ($VSS = 1096 \text{ mgVSS/l}$).

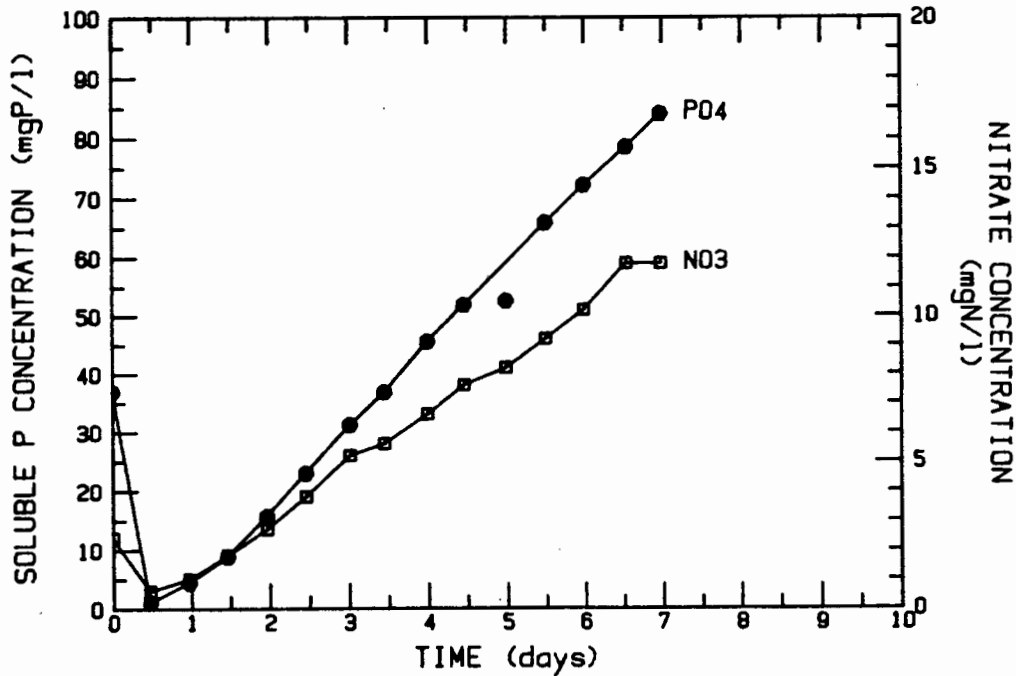


Fig 7.6(b): Total soluble phosphate (PO_4) and nitrate (NO_3) concentration-time profiles for the batch aerobic digestion in Fig 7.6(a).

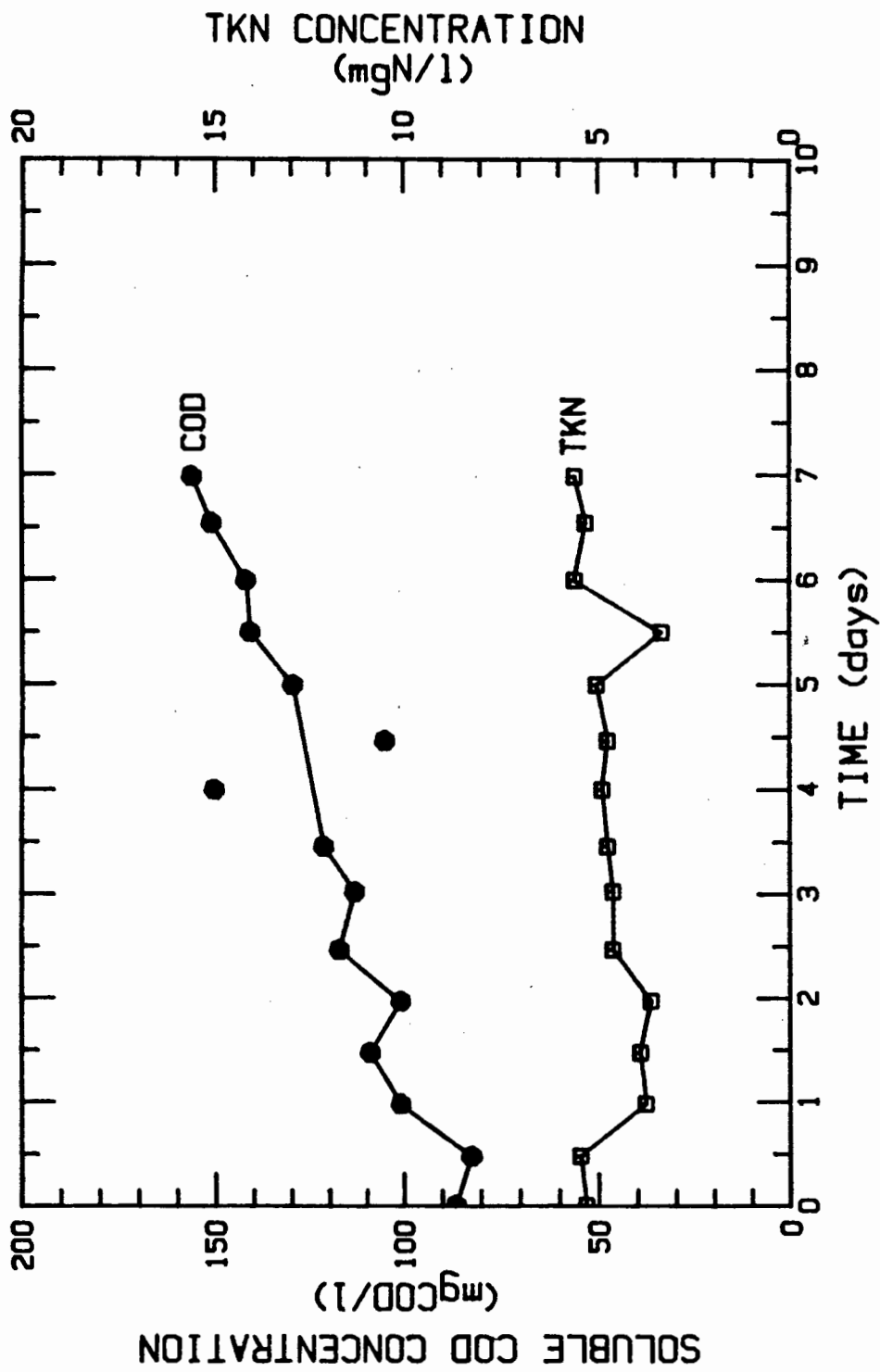


Fig 7.6(c): Soluble COD and TKN concentration–time profiles for the batch aerobic digestion in Fig 7.6(a).

during the course of the experiment. The effect of nitrate (NO_3) was eliminated by keeping the batch unaerated until the nitrate was depleted before adding the acetate. The pH in the batch was continuously monitored and maintained in the region 7,4 to 7,6. At intervals samples were taken from the batch and filtered immediately. The filtrate was analyzed for total P, acetate and NO_3 concentrations. (In all these tests NO_3 concentrations were zero). A series of tests was undertaken with serially increasing additions of acetate per mgVSS. The following observations were made:

- (1) With relatively low acetate addition, i.e. 0,11 mgCOD acetate/mgVSS, (see Fig 7.7), the disappearance of acetate is linear, so also the release of P. This implies that the reactions are independent of the acetate concentration and probably dependent only on the polyP organism concentration. When the acetate is depleted, P release continues, but at a greatly reduced rate (see also Chapter 3).
- (2) With relatively high acetate addition, 0,265 mgCOD acetate/mgVSS, (see Fig 7.8), while acetate is present both the disappearance of the acetate and the release of P show a two phase linear relationship. The first phase conforms to that in (1) above. The rate in the second phase is slower than that in the first phase (about half) and shows a different stoichiometric ratio between P released and acetate taken up. The point in the test at which the change occurs is not easily determined from the concentration-time profiles, but can be more clearly distinguished by plotting the time paired data of acetate and P against each other (see Fig 7.9). From a series of such tests on a particular mixed liquor it was found that the first linear phase operates for acetate addition up to approximately 0,14 mgCOD/mgVSS. With higher additions of acetate the first phase operates until approximately 0,14 mgCOD/mgVSS acetate has been sequestered, thereafter the second phase is observed. In such instances after all the acetate has been sequestered, again the P release continues, but at the greatly reduced rate as in (1) above.
- (3) With excess acetate addition, the second phase of acetate uptake also terminates, even though acetate is still available. At this termination point it appears that all the P that can be released has been released, (see Fig 7.10). After the termination there is only a slight further release of P with time. From these tests the fraction of P that can be released with excess acetate addition was approximately 70 percent of the P contained in the batch sludge. This fraction probably corresponds to the low molecular

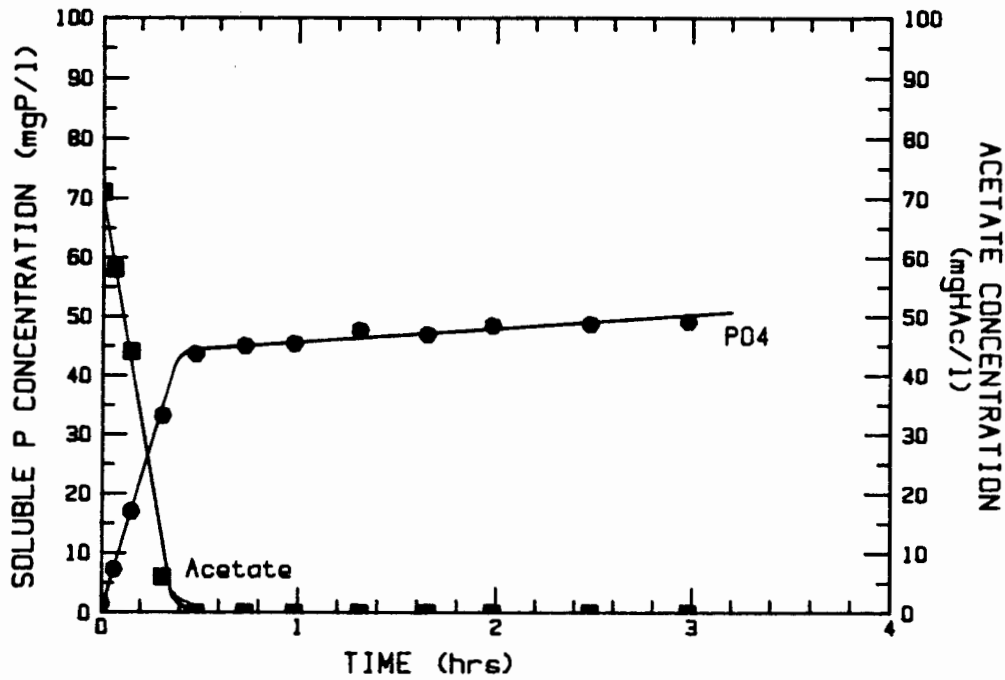


Fig 7.7: Total soluble phosphate (PO₄) and acetate concentration–time profiles with anaerobic addition of 0.11 mgCOD acetate/mgVSS to a mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 684 mgVSS/l).

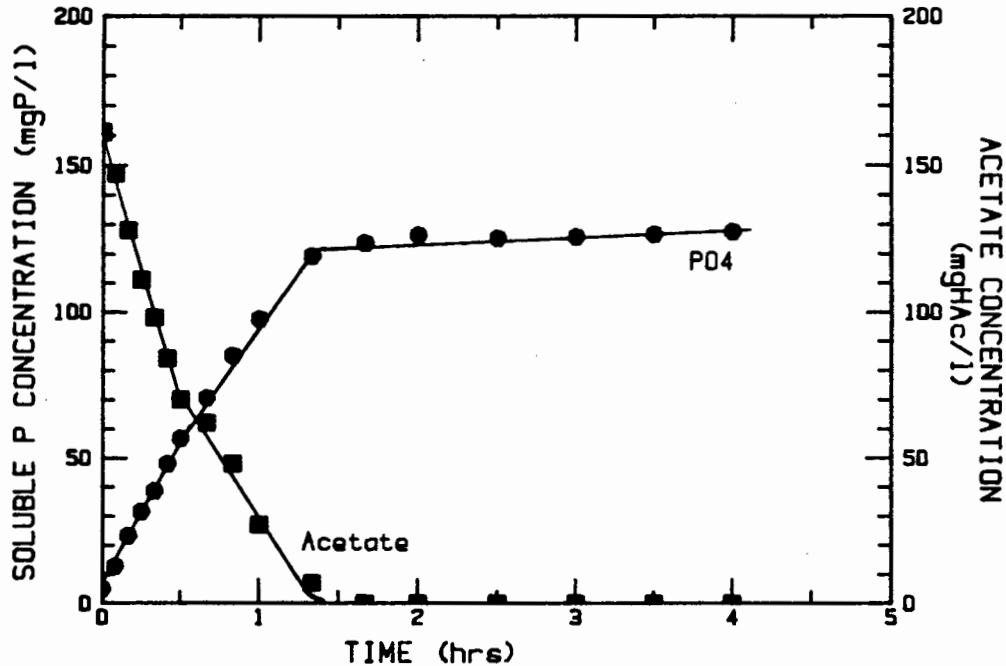


Fig 7.8: Total soluble phosphate (PO₄) and acetate concentration–time profiles with anaerobic addition of 0.265 mgCOD acetate/mgVSS to a mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 651 mgVSS/l).

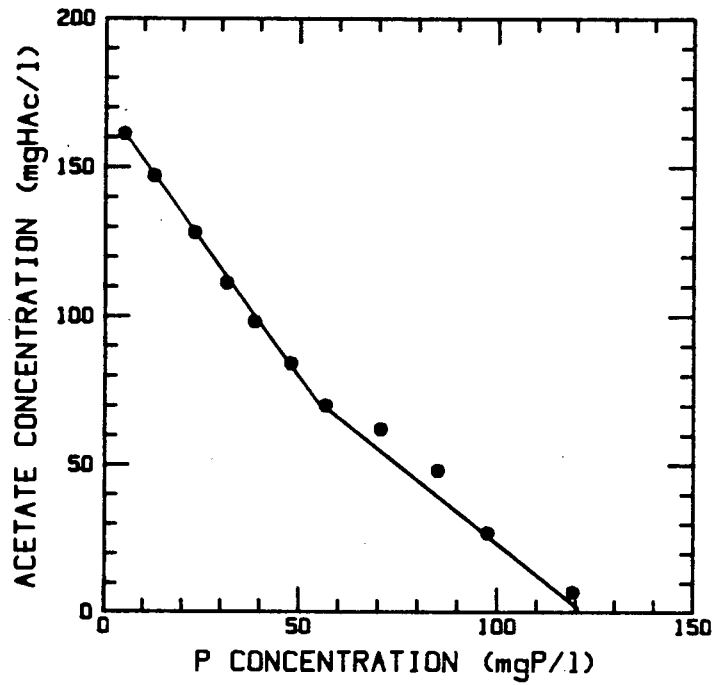


Fig 7.9: Plot of acetate concentration versus total soluble phosphate concentration for the time paired data in Fig 7.8.

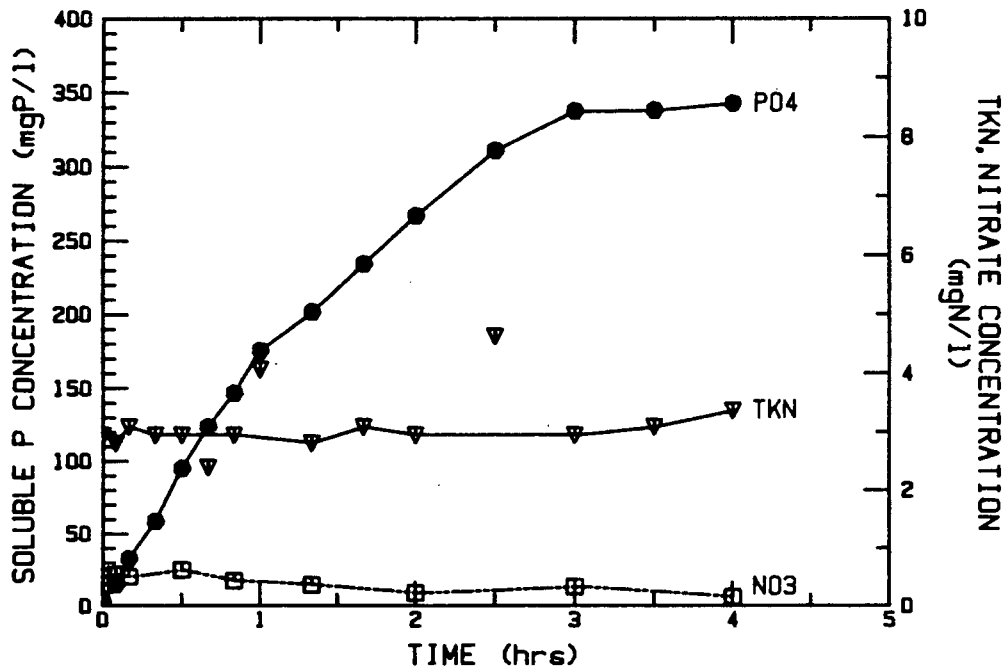


Fig 7.10: Experimentally observed total soluble phosphate (PO₄), TKN and nitrate (NO₃) concentration-time profiles with anaerobic addition of excess acetate (0.9 mgCOD acetate/mgVSS) to a mixed liquor batch drawn from the Bardenpho enhanced culture system.

weight polyP identified in the organism mass by Mino *et al.* (1984). They also identified a high molecular weight polyP but they found that this was not released. Their studies were conducted using mixed cultures so that it is not clear whether the two polyP types were present in the same organism, or in different organism species.

In the batch studies the fraction of the P content of the mixed liquor that was released (up to 70 percent), is greatly in excess of the fraction usually released in the anaerobic reactors of modified Bardenpho and UCT enhanced culture systems. In these systems the acetate addition per mgVSS in the anaerobic reactor is so low that only about 13 percent of the P content of the VSS is released (Tables 7.2 and 7.3), i.e. about 20 percent of the P that *can* be released. Thus, the extreme conditions imposed in the batch tests are unlikely to be replicated in the P removal systems – generally in these systems, the P release will be such that it remains in the first linear release phase.

For the series of tests with acetate addition as in (1) to (3) above, the rates of acetate uptake and P release, and the stoichiometric ratios between the P released and the acetate taken up for both phases of P release were determined. The values for the first phase are shown plotted on normal probability paper in Fig 7.11(a and b) respectively. From these plots and similar plots for the second phase, the mean values for the rates and the ratios were determined and are listed in Table 7.4. With regard to the stoichiometric ratio for the first phase, it is 1,08 mole P released/mol acetate taken up or equivalently 0,52 mgP/mgCOD sodium acetate. This value is in close conformity with that predicted by the biochemical model (see Chapter 5) and also agrees with observations on P removal systems treating municipal wastewater, see Chapter 3.

4.3 Aerobic response after P release

This series of batch tests was conducted to investigate the aerobic response following anaerobic P release. Mixed liquor samples were drawn from the last aerobic reactor of the enhanced culture systems and placed in continuously stirred batch reactors. The surface of the mixed liquor was covered, as described previously. The batch was aerated continuously for approximately 24 hours to utilize any stored PHB carried over from the steady state systems. During this period OUR, total soluble P, TKN and NO_3 concentrations were monitored and the pH controlled, as described previously. After 24 hours the air was switched off and nitrogen gas was continuously bubbled through the mixed liquor to strip off oxygen and to prevent

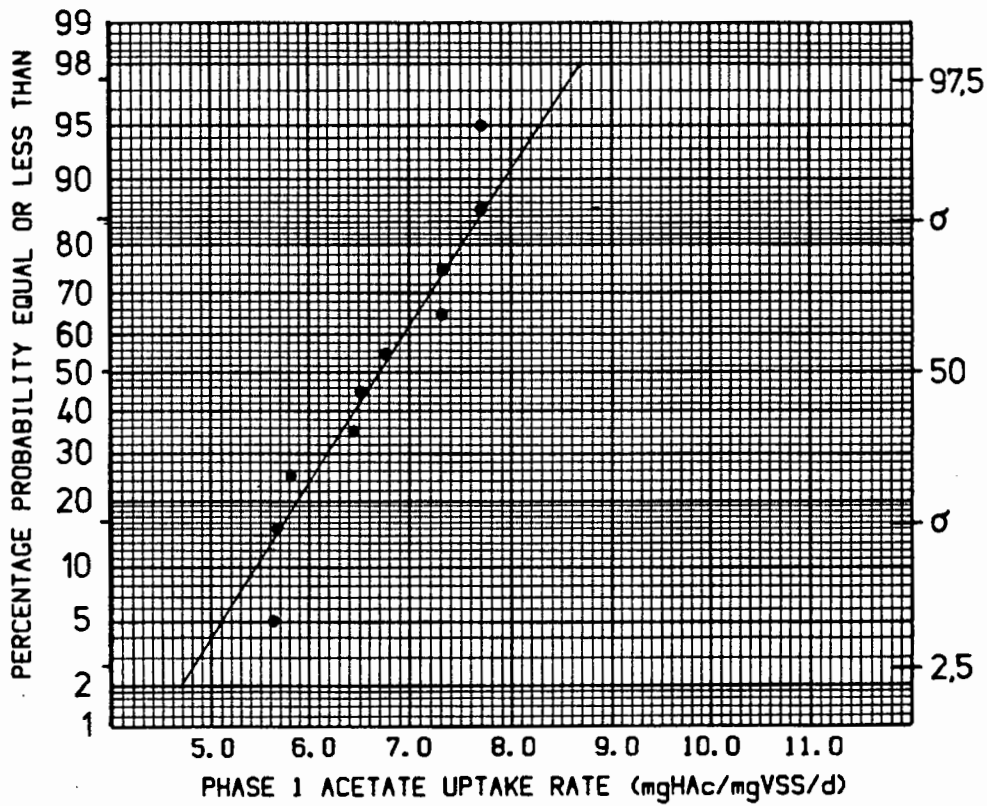


Fig 7.11(a): Statistical plot of first phase rates of acetate uptake in anaerobic batch tests such as in Fig 7.7.

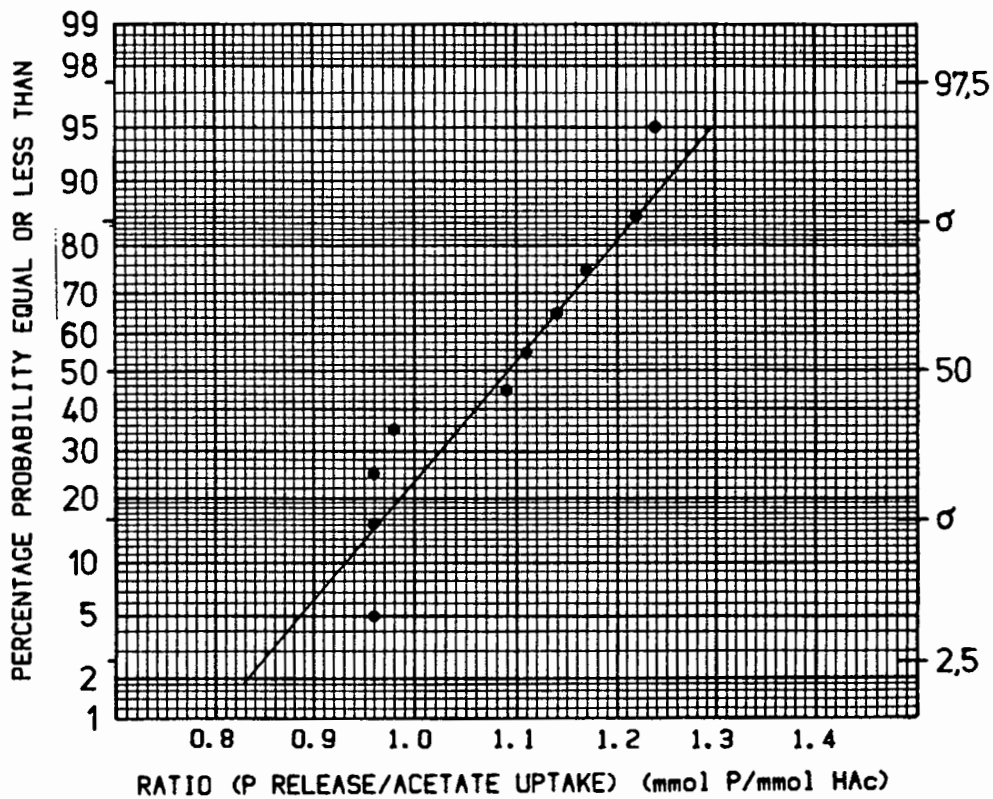


Fig 7.11(b): Statistical plot of first phase stoichiometric ratio between P release and acetate uptake.

Table 7.4: Rate of acetate uptake, P release and the ratio P released/acetate uptake, from anaerobic batch tests on enhanced culture sludges.

Rate of acetate uptake (mgCOD*/mgVSS/d)		Rate of P release (mgP/mgVSS/d)		Ratio P release/acetate uptake (mgP/mgCOD*)**	
Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
7,17	3,11	3,73	2,74	0,52	0,88

* To convert to mg HAC divide mg COD by 1,07

** To convert to mmol P divide mg P by 30,97, to mmol acetate divide mg COD by (60.1,07)

oxygen ingress. Total soluble P, TKN and NO_3 concentrations were monitored and pH controlled as before. This state was maintained until the NO_3 concentration reduced to zero, whereupon a selected mass of standard substrate and nutrients (sodium acetate, yeast extract, K_2HPO_4 , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and NH_4Cl , Chapter 6) were added. The batch was maintained in an anaerobic state by bubbling nitrogen gas through the mixed liquor. Total soluble P, TKN, NO_3 and acetate concentrations were monitored and pH controlled as before. The acetate concentration reduced to zero during this time. (This period corresponds to that described in section 4.2 above, i.e. anaerobic response with acetate addition). After the acetate reduced to zero the air was switched on and the OUR, total soluble P, TKN and NO_3 concentrations were monitored and pH controlled, as before.

In this series of batch tests the focus of interest was on the final aeration period. The relevant responses observed for two typical tests are shown plotted in Figs 7.12 and 7.13 for aeration following anaerobic P release with acetate addition of 0,207 and 0,363 mgCOD/mgVSS respectively. To eliminate the confounding effects of OUR for nitrification, this OUR was estimated from the increase in NO_3 in the test and subtracted from the observed OUR to give the OUR for carbonaceous material oxidation only. It is this carbonaceous OUR that is shown in the Figures.

Three features in the response curves are to be noted:

- (1) The OUR curves indicate a saturation type reaction: With the lower addition of acetate to the anaerobic phase, the OUR shows a declining magnitude from the start of aeration (Fig 7.12) whereas with higher addition of acetate, the OUR exhibits an initial plateau and thereafter declines (Fig 7.13). This behaviour implicates a Monod type of relationship between the specific substrate utilization (or growth) rate and the substrate concentration stored internally (as PHB) per unit of volatile active mass (polyP organisms).
- (2) The change in P concentration appears to be linked to substrate utilization: Initially, (see Fig 7.13), the P concentration drops linearly with time (< 5 hrs) thereafter the change in P concentration exhibits a first order type of reaction with respect to itself. This behaviour conforms to that expected of the substrate concentration in the reactions governed by a Monod type of relationship. Since internally stored acetate (as PHB) forms the substrate and not the P, an assumption that links PHB utilization directly with P

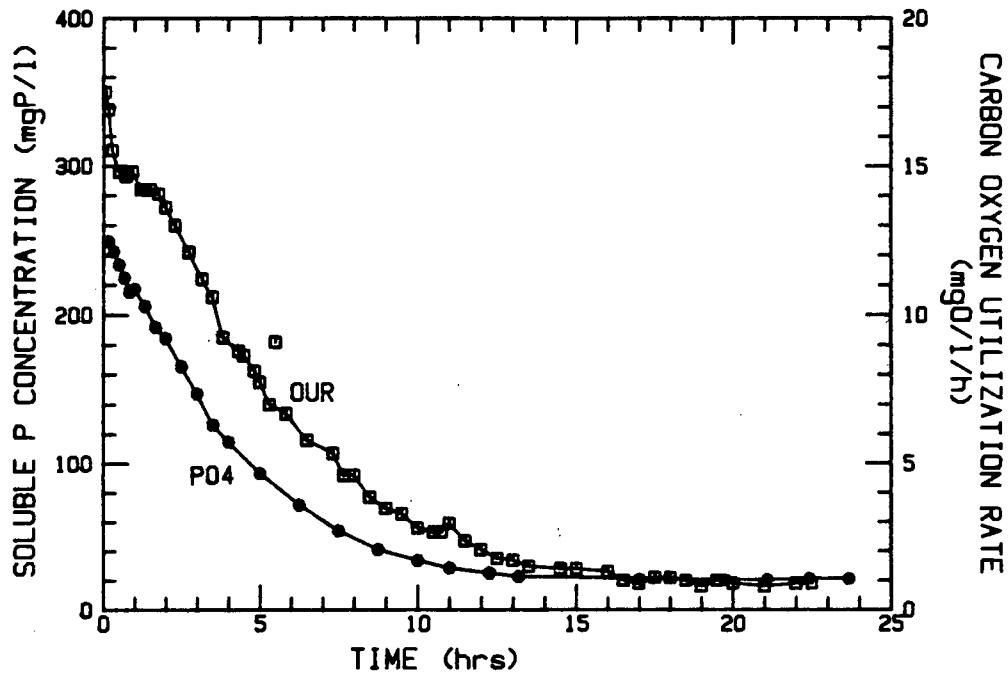


Fig 7.12: Total soluble phosphate concentrations (PO₄) and carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0,207 mgCOD acetate/mgVSS to mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 1041 mgVSS/l).

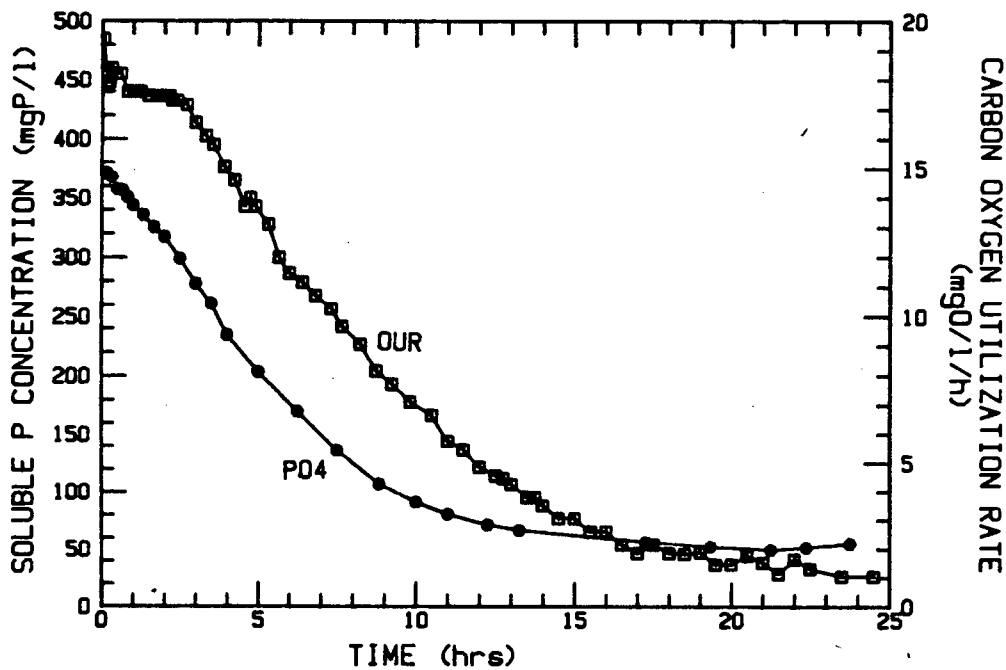


Fig 7.13: Total soluble phosphate concentrations (PO₄) and carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0,363 mgCOD acetate/mgVSS to mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 1100 mgVSS/l).

uptake for polyP formation seems reasonable.

- (3) When the OUR levels off, the P concentration commences to increase, indicating the start of the endogenous digestion phase.

A further aerobic batch test, following anaerobic acetate addition of 0,215 mgCOD/mgVSS [as in section 4.2(2) above], was conducted in which there was not sufficient soluble P for uptake. The P and carbonaceous OUR responses are shown plotted in Fig 7.14. Three features in this test are to be noted:

- (1) The initial stage of the test shows the OUR decreasing from the start indicating unsaturated internally stored PHB conditions similar to Fig 7.12.
- (2) After about six hours the soluble P concentration reaches zero. When the P concentration reaches zero there is a precipitous drop in the OUR.
- (3) The P concentration continues to remain close to zero, and the OUR continues to decrease but at a significantly reduced rate compared to the initial stage, but greater than that associated with endogenous mass loss.

The results of this batch test indicate that when P becomes limiting a qualitative change in the polyP organism behaviour takes place: It appears that stored substrate utilization continues, but at a reduced rate and without the associated P uptake for polyP formation. Necessarily the P requirements for normal cell synthesis would be supplied from the polyP pool. This behaviour of the polyP organisms has been noted by van Groenestijn and Deinema (1985).

A final aerobic batch test, following anaerobic acetate addition of 0,135 mgCOD/mgVSS, was conducted to investigate the utilization of nitrate as a nitrogen (N) source for cell synthesis. From the steady state response of the enhanced culture systems, described previously, it was concluded that nitrate can serve as a N source for synthesis. However, the steady state system data provided no kinetic information as to differences that may arise when utilizing nitrate as opposed to ammonia as a nitrogen source (e.g. a change in growth rate). Accordingly it was proposed to investigate changes in kinetic behaviour when the nitrogen source is changed from ammonia to nitrate.

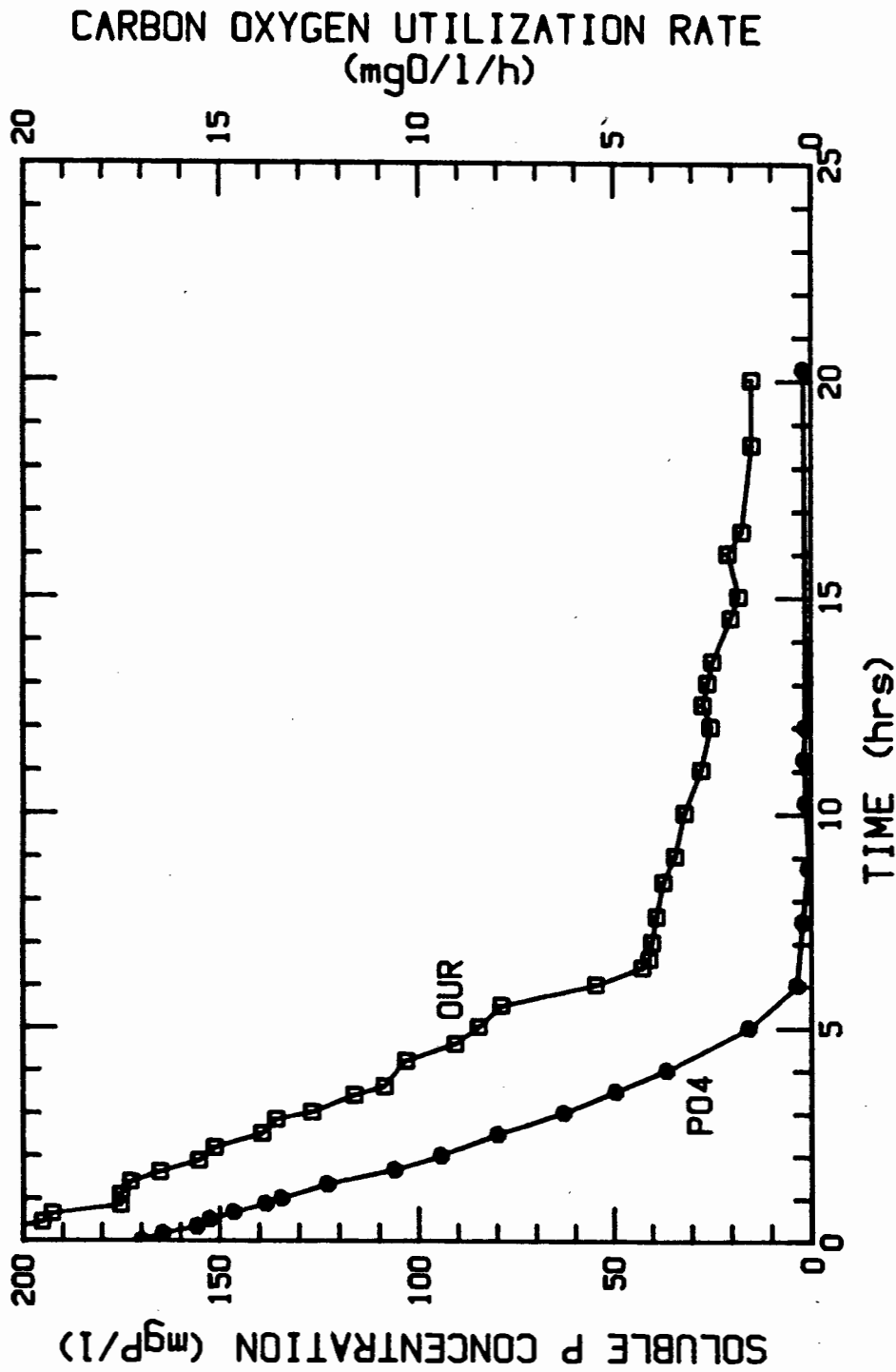


Fig 7.14: Total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0.22 mgCOD acetate/mgVSS to mixed liquor batch drawn from the Bardenpho enhanced culture system. The PO_4 concentration falls to zero during the course of this test (VSS = 1226 mgVSS/l).

A batch of mixed liquor was drawn from the last aerobic reactor of the enhanced culture system and placed in a continuously stirred batch reactor. The same procedure as described above was followed, with the following exceptions: Less ammonia was added with the acetate at the start of the anaerobic period; during the subsequent period while substrate still was being utilized *inter alia* for synthesis, it was envisaged that the ammonia would reduce to zero enabling the change in kinetic behaviour to be monitored. At the start of the aeration period 5 mgN/l batch volume of NaNO_3 was added to ensure sufficient nitrate would be present. The response profiles for the aeration period following anaerobic acetate addition of 0,135 mgCOD/mgVSS are shown plotted in Fig 7.15(a and b). The following features in the response curves are to be noted:

- (1) During the initial phase of the aeration period (0-5,5 hrs), TKN is present and the OUR and P concentration profiles correspond to those in Fig 7.12. The TKN concentration declines during this period due to utilization of ammonia for nitrification and as a N source for cell synthesis. The nitrification reaction is reflected in the increase in the NO_3 concentration.
- (2) When all the available TKN has been utilized (after approximately 5,5 hrs), ammonia no longer is available as a N source for synthesis and the NO_3 concentration commences to decrease indicating utilization of nitrate as a N source for synthesis. This observation verifies the steady state system observations.
- (3) When the change occurs, from ammonia to nitrate as a N source for synthesis, no observable change in the carbonaceous OUR or P concentration profiles are apparent. This was verified as follows: The P concentration decreases with time eventually to a stable value ($X = 48,24 \text{ mgP/l}$). This value is subtracted from the observed P concentration during the period of change and the difference plotted $\log(\text{P concentration}-X)$ versus time (Fig 7.16); the plot is linear. This would indicate that utilization of NO_3 as opposed to ammonia as a nitrogen source does not affect the kinetic behaviour of polyP organism growth reactions.

4.4 Anoxic response after P release

It was concluded earlier, from data obtained on the steady state enhanced culture systems, that in these systems, denitrification by the polyP organisms is minimal. Accordingly batch tests were conducted to investigate this aspect further.

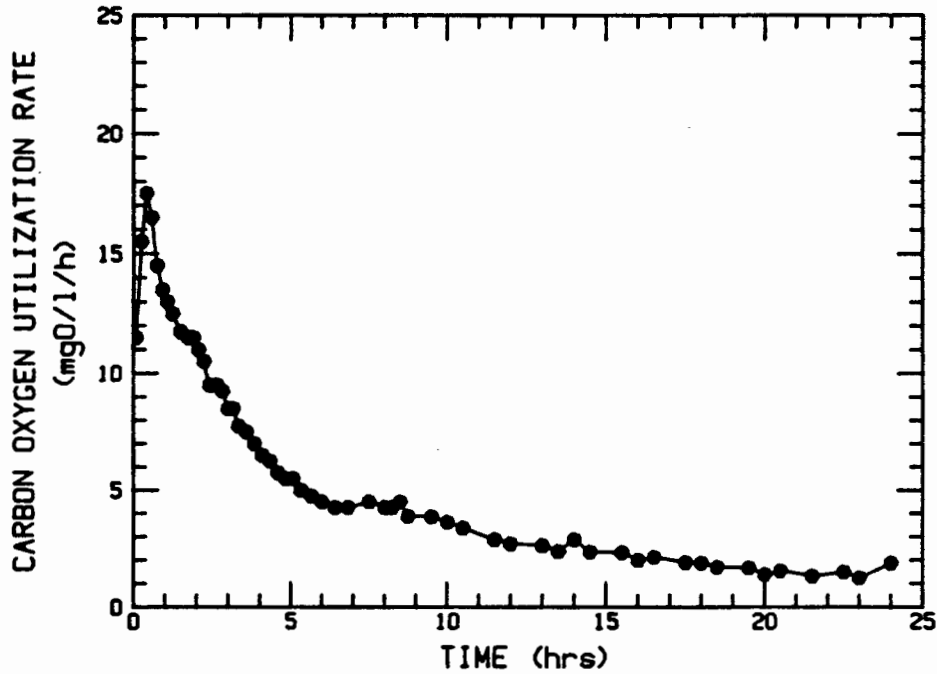


Fig 7.15(a):

Carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0,125 mg acetate/mgVSS to mixed liquor batch drawn from enhanced culture system. The TKN concentration falls to a steady value during the course of this test (VSS = 1143 mgVSS/l).

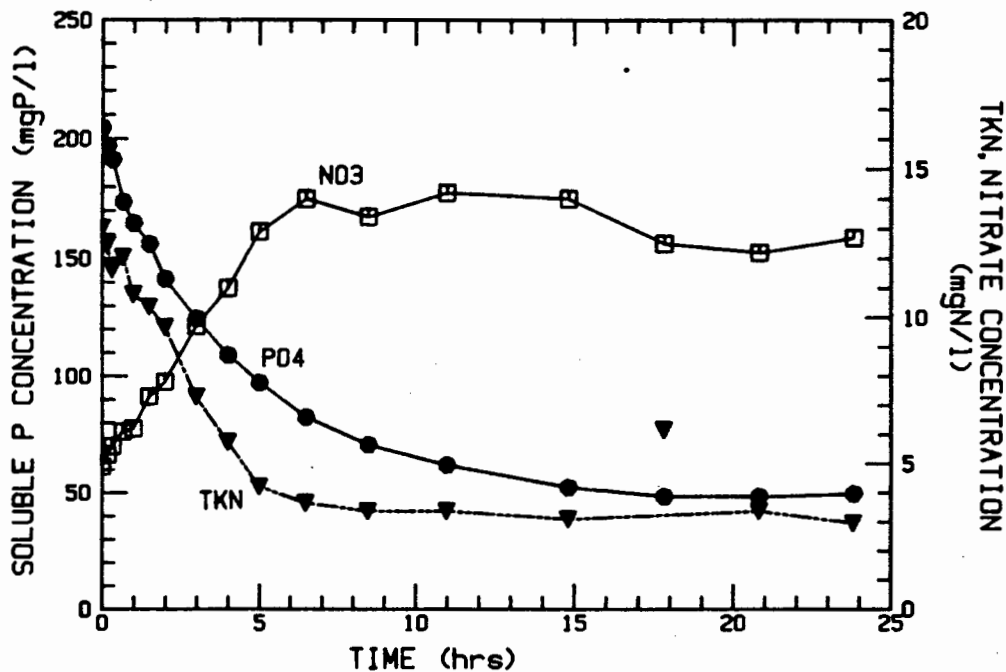


Fig 7.15(b):

Total soluble phosphorus (PO_4), TKN and nitrate (NO_3) concentration-time profiles for the batch test in Fig 7.15(a).

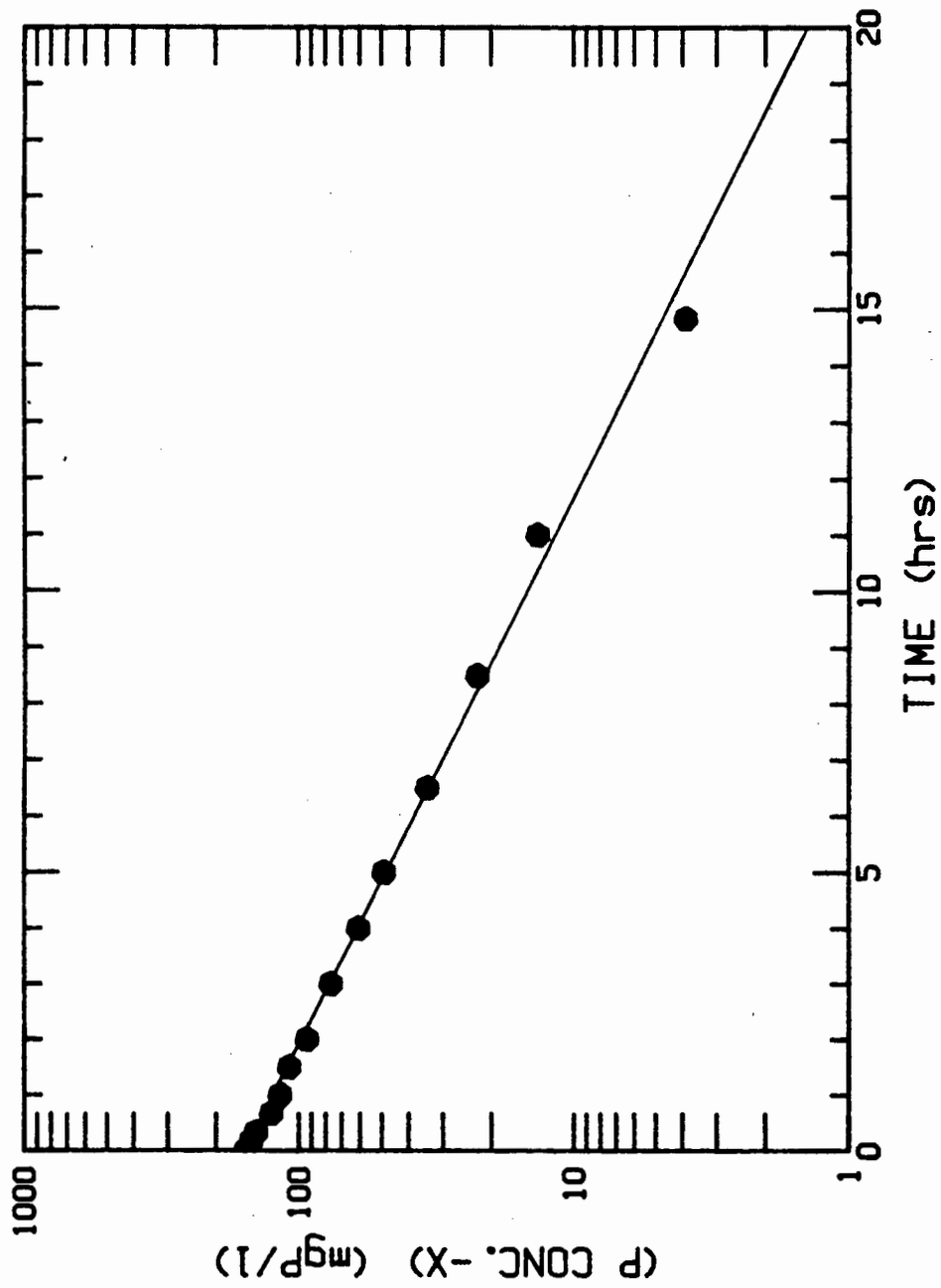


Fig 7.16: Semilog plot of total soluble phosphorus (PO_4) concentration versus time for the data in Fig 7.15(b) ($X = 48,24$).

Mixed liquor was drawn from the anaerobic reactor of the enhanced culture system. Analysis of acetate in the anaerobic reactor had shown the concentration to be zero, indicating that all the acetate had been sequestered, i.e. no acetate was carried over to the batch test. The mixed liquor was placed in a sealed batch reactor and continually sparged with nitrogen gas to prevent oxygen ingress into the mixed liquor. At the start of the test samples were drawn for VSS and TSS determinations. At time zero, $\pm 20 \text{ mgN/l}$ as KNO_3 was added to the batch. During the course of the test total P, NO_3 , NO_2 , COD on filtered samples and intracellular PHB concentrations were monitored and the pH controlled, as described previously. The relevant response curves are shown plotted in Fig 7.17 (a and b). The following observations were made:

- (1) The nitrate concentration slowly decreased during the course of this test, in a linear fashion, due to denitrification. The rate of denitrification was determined to be $0,043 \text{ mgN/mgVSS/d}$. This rate is very much lower than those measured for mixed culture activated sludge systems (van Haandel *et al.*, 1981).
- (2) Nitrite concentration increased very slowly during the course of the batch test. Approximately 40 percent of the nitrate was reduced to nitrite only. This is in conformity with the findings in Chapter 4 where it was shown that a number of *Acinetobacter*, a polyP organism, strains can reduce nitrate to nitrite only.
- (3) The P concentration showed minor *increase* during the test. This would indicate that, in the enhanced cultures, insignificant P uptake occurs with nitrate as external electron acceptor.
- (4) The intracellular PHB concentration remained approximately constant. This would indicate that PHB is not being utilized as a substrate source for denitrification.
- (5) The soluble COD concentration increased slightly during the course of the test indicating accumulation of soluble unbiodegradable COD, as noted earlier.

The data from this batch test supports the conclusion, drawn from the steady state systems, that denitrification by the polyP organisms (and hence associated P uptake

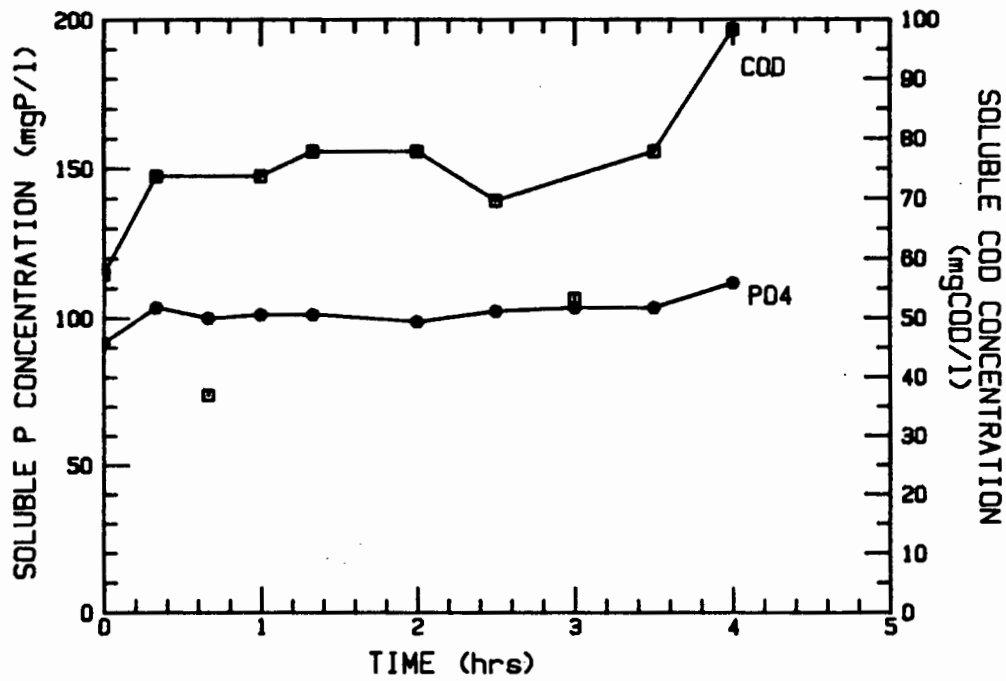


Fig 7.17(a): Total soluble phosphorus (PO_4) and COD concentrations observed on addition of KNO_3 to batch of mixed drawn from the anaerobic reactor of the enhanced culture system ($\text{VSS} = 594 \text{ mgVSS/l}$).

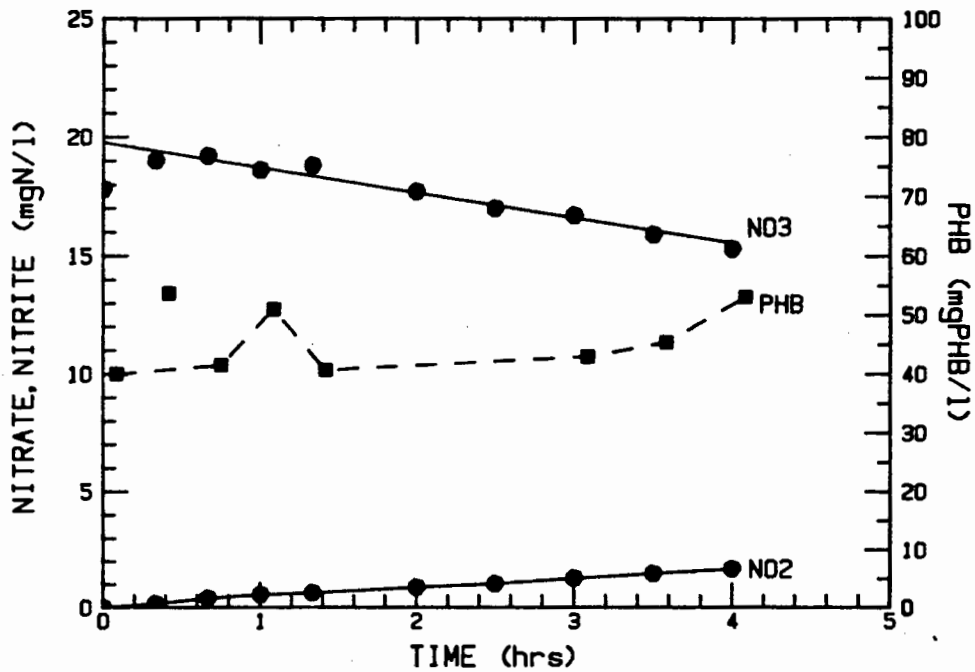


Fig 7.17(b): Nitrate (NO_3), nitrite (NO_2) and intracellular PHB concentration-time profiles for the batch test in Fig 7.17(a).

potential) in the enhanced cultures is negligible, i.e. denitrification can be neglected when developing a kinetic model to describe the behaviour of such systems.

4.5 Acetate addition under aerobic conditions

Mixed liquor samples were drawn from the last aerobic reactor of the enhanced culture systems and placed in batch reactors. As described previously, the surface of the batch mixed liquor was covered with floating plastic balls. The batch was aerated and OUR, total soluble P, TKN, COD and NO_3 concentrations monitored and pH controlled as before. When all the stored PHB had been depleted (judged from P concentration and OUR response, i.e. after about 15 hours in Figs 7.12 and 7.13) acetate was added and aeration continued as before. The response curves following acetate addition are shown in Fig 7.18(a and b). The interesting features are:

- (1) The acetate disappears at a fast rate with an associated *release* of P. This behaviour appears similar to that with acetate addition under anaerobic conditions, i.e. P release associated with acetate uptake and storage as PHB.
- (2) Initially, the OUR is high and remains high until the acetate concentration becomes zero.
- (3) The P concentration increases but at a declining rate to reach a maximum concentration that appears to coincide with the complete removal of acetate. Thereafter, the P concentration declines as described in the aerobic tests in section 4.3 above.
- (4) The OUR drops *precipitously* when the acetate concentration reaches zero. If the polyP organisms utilize only stored PHB for growth, then a precipitous OUR decrease is not expected because the stored PHB remains high at the time the acetate is depleted. The precipitous decrease in OUR, therefore, would indicate that the polyP organisms also utilize acetate directly for growth under aerobic conditions, while simultaneously taking up acetate, storing it as PHB and releasing P.
- (5) Oxygen utilization continues at a rate higher than that for endogenous respiration after the acetate concentration becomes zero. This indicates a utilization of stored PHB, as described in the aerobic tests in section 4.3 above.

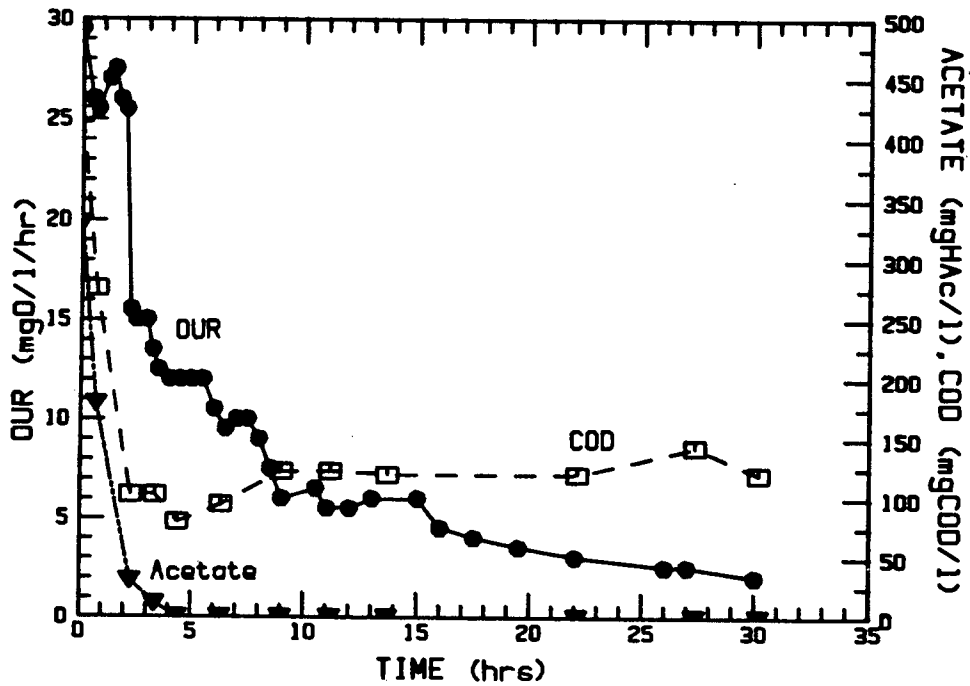


Fig 7.18(a): Total oxygen utilization rate (OUR), COD and acetate concentration–time profiles observed on aerobic acetate addition of 0,324 mgCOD acetate/mgVSS to a batch of mixed liquor drawn from the Bardenpho enhanced culture system. (VSS = 1080 mgVSS/l).

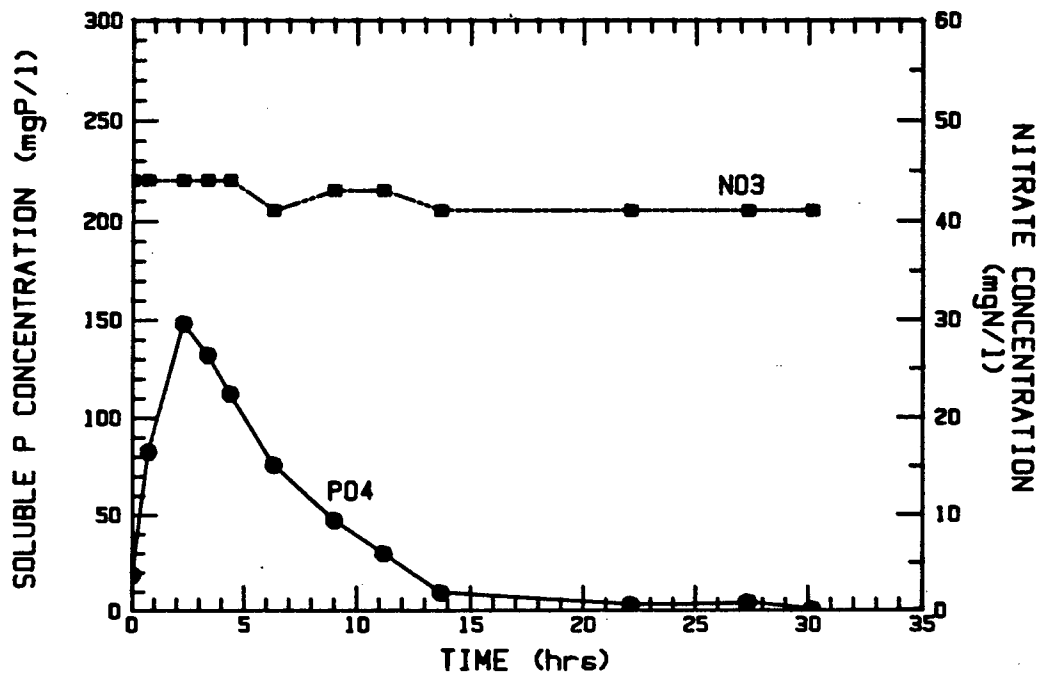


Fig 7.18(b): Total soluble phosphate (PO₄) and nitrate (NO₃) concentrations observed on aerobic acetate addition of 0,324 mgCOD acetate/mgVSS to a batch of mixed liquor drawn from the Bardenpho enhanced culture system (VSS = 1080/l).

- (6) The soluble COD initially *decreases*, due to acetate uptake, but thereafter slowly increases. This behaviour appears to indicate that during endogenous respiration there is a generation of "unbiodegradable" soluble COD, as noted before.

This test verifies the prediction of the biochemical model that acetate storage as PHB with P release can take place under aerobic conditions, i.e. an anaerobic state is not a prerequisite to obtain P release with acetate addition. This however does not imply that an aerobic system could be operated with acetate as influent to give biological excess P removal.

4.6 Cation release and uptake

In the development of the steady state enhanced culture systems, it was found that adequate quantities of magnesium (Mg) were essential for the successful operation of biological excess P removal systems (see Chapter 6). It was hypothesized that the function of Mg is to serve as the principal counterion stabilizing the polyP chain. Consequently, the pattern of Mg release and uptake should be closely related to the P release and uptake. Accordingly, it was decided to investigate this aspect further.

For the batch tests described above in sections 4.1, 4.2 and 4.3 the samples also were analyzed for the cations magnesium (Mg), calcium (Ca), potassium (K) and sodium (Na). For each batch test the cation concentration was plotted against the P concentrations. From these plots the ratio cation released/taken up per P release/taken up can be calculated. The mean values obtained from all the results are listed in Table 7.5 together with similar results obtained by other research groups. The results obtained in this investigation are in agreement with those obtained by other research groups, with the exception of Na: In this study no association was found between Na and P release/uptake but Fukase *et al* (1982) found a significant association. Very little Ca release/uptake accompanies the P release/uptake, 0,05 mol Ca/mol P. This excludes the possibility that calcium phosphate dissolution/precipitation respectively as the cause for the observed changes in P concentration. Both Mg and K are released and taken up in concert with P release and uptake. Probably, both these ions stabilize the polyP chains by acting as counterions. The ratio (total mol + ve released)/(mol P released) is 0,92 which conforms reasonably to the ratio of 1,0 predicted by the biochemical model.

5. DISCUSSION

It is most unlikely that a model can be developed that describes a physical

Table 7.5: Ratio of cations released, or taken up, to P released, or taken up.

Research group	(Cation release/uptake)/(P release/uptake) (mmol cation/mmol P)				Charge balance (mmol + ve/ mmol P)
	Mg	K	Ca	Na	
Fukase <i>et al.</i> (1982)	0,28	0,31	0,05	0,12	0,97
Arvin <i>et al.</i> (1985)	0,32	0,23	-	-	0,87
Comeau <i>et al.</i> (1985)	0,24	0,34	0,06	-	0,94
This study	0,26	0,30	0,05	0	0,92

*The charge balance is the ratio of the sum of the positive charges released or taken up to P released or taken up.

phenomenon completely; theoretically a complete description should include aspects down to the most fundamental level. To take an extreme example, in the description of a biosystem, for completeness DNA molecule behaviour should be included. Instead less complete models are developed, at some level of organization (Odum, 1971); only characteristics important at that level are incorporated in the model description. Success in modelling greatly depends on the ability to identify the level of organization and the principal or relevant characteristics controlling the phenomenon at that level. For example, one cannot directly implicate the biochemical control mechanisms of P release and uptake as described in the biochemical model (such as ADP/ATP and NAD/NADH ratios) but rather parameters are implicated that appear to be important at the level of interest, in this instance the mass behaviour of a *population* of selected organisms. Monod's equation is a typical example, it relates the rate of growth of an organism mass directly to substrate concentration whereas at a lower level of organization, the molecular level, the growth rate is controlled also by ADP/ATP and NAD/NADH ratios, amongst others.

As stated earlier, to set up a mathematical model that describes the stoichiometry and kinetic behaviour of the biological excess P removal phenomenon it is required to;

- (1) identify the essential compounds utilized or formed,
- (2) identify the processes acting on these compounds,
- (3) conceptualize a qualitative mechanistic model of the behaviour and,
- (4) formulate mathematically the process rates, stoichiometry and transport relationships.

In this chapter only (1) to (3) are considered; the mathematical model will be set out in Chapter 8.

5.1 Compounds

There appear to be 12 essential compounds *directly* involved in biological excess P removal. Some of these compounds are directly observable whereas with others, their existence has to be inferred, either from hypothesized biochemical behaviour or from the requirement of mass balances, because the means for measuring these

compounds have not been available in the laboratory, or, where the means have been available, these have not been sensitive enough to give quantitative parameters. Compounds directly observable are concentrations of acetate, soluble P, oxygen, nitrate, ammonia, readily biodegradable COD and unbiodegradable soluble COD. Compounds inferred are polyphosphate (polyP), polyhydroxybutyrate (PHB), apparently soluble unbiodegradable organic N, endogenous particulate residue and polyP organisms.

5.2 Processes

The processes that act on the compounds above are identified by observing changes in the compounds under a variety of conditions. In this manner, eventually, 13 essential processes have been identified.

5.3 Conceptual model

From the experimental investigation it is evident that the processes that act on the compounds can be grouped into three broad categories - (1) sequestration of acetate, (2) growth, and (3) endogenous mass loss. Description of the conceptual model is facilitated if each of the three categories are developed separately.

- (1) Sequestration of acetate: Sequestration can take place under anaerobic or aerobic conditions. This is illustrated in Figs 7.7, 7.8 and 7.10 and 7.18 (a and b) respectively.

In the anaerobic situation, the compound acetate is taken up and stored as PHB. The rate of acetate sequestration can be observed directly and appears to be zero order with respect to the acetate concentration, as observed by the linearities in the acetate-time plots, Figs 7.7, 7.8 and 7.10. The mean specific rate estimates (i.e. rate/volatile solids) for the two phases of sequestration from a number of anaerobic sequestration batch tests on mixed liquor from the 20 day sludge age enhanced culture system are given in Table 7.4. Concomitant with acetate sequestration, stored polyP is cleaved and P released to the bulk liquid, causing the observable increase in soluble P concentration, see Figs 7.7, 7.8 and 7.10. There appear to be three phases of sequestration; in the first phase the mass of P released appears to be proportional to the mass of acetate taken up, see mean values in Table 7.4; apparently only a fraction of the polyP can be released at this first rate, approximately 30 percent. Should acetate still be available when this polyP

fraction is exhausted, a second rate of acetate uptake appears to operate; not only is this rate of acetate uptake slower than the first rate, but also the mass of P released per mass acetate stored is higher, Fig 7.8 and Table 7.4. To this second phase also there appears to be a limit, probably the mass of polyP available. If acetate still is present at the end of the second phase, sequestration of acetate appears to cease, but a very low rate of P release still is apparent, see Fig 7.10, most likely due to death of organisms (see endogenous mass loss discussion below). Should the acetate concentration become zero during either the first or the second phase of P release then both types of sequestration cease and a third phase of P release occurs; in this phase P is released without acetate uptake and storage (no acetate being available, see Figs 7.7 and 7.8). It is hypothesized that the third phase of P release is one due to polyP cleavage for maintenance energy requirements under anaerobic conditions (see endogenous mass loss discussion below).

The second phase of P release, and thus the potential release of all the polyP, is unlikely to occur in the steady state continuous flow enhanced culture systems because the substrate loading rates per unit mass of polyP organisms are much less than the loading rates that can be imposed in a batch test; usually only the first sequestration phase will be encountered. In system operation, second phase P release might occur only under injudiciously high acetate load increments in system startup, or under extreme cyclic loading. Normally this would be true also for mixed culture excess P removal systems.

In the aerobic situation the batch tests indicate that sequestration of acetate as described for the anaerobic situation, also takes place, Fig 7.18(a and b). However, the rates of aerobic sequestration cannot be observed directly because three phenomena take place at the same time:

- (i) Acetate uptake for storage as PHB with associated P release.
- (ii) Direct uptake and utilization of acetate for growth without P release or uptake.
- (iii) Utilization of stored PHB with associated P uptake.

Normally in enhanced culture and mixed culture excess P removal systems, significant concentrations of acetate in aerobic reactors are unusual and aerobic sequestration is unlikely. From Chapter 6 it would appear that in enhanced cultures, where acetate does pass into the aerobic section (due to malfunction or inappropriate operation), the specific rate of acetate uptake of the non-polyP organisms (that may be present) is higher than that for the polyP organisms, and the non-polyP organisms tend to increase their concentration. Should the acetate 'leakage' continue, a slow decline of polyP organism growth takes place, leakage of acetate increases and the enhanced culture collapses. The instability inherent in this behavioural pattern makes that it is not possible to model aerobic acetate sequestration. For modelling purposes a limitation must be imposed: It is assumed that the anaerobic phase is sufficient to sequester all the available acetate.

- (2) Growth: Growth processes require the presence of an external electron acceptor. The external electron acceptors for polyP organism growth usually are oxygen (Juni, 1978) or nitrate (Lötter, 1985). However, in the enhanced culture systems, the conditions did not induce growth of denitrifying polyP organism species (or did so only slightly). This is illustrated from the steady state results, Tables 7.2 and 7.3, and in the batch test, Fig 7.17. As a consequence, no information on the behaviour of the denitrifying polyP organism species could be obtained. Hence, in this model, denitrification by polyP organisms will not be included. (However, effort in this regard should not be abandoned because there is strong evidence that there are polyP organism species that do use nitrate, with significant growth and polyP accumulation, e.g. Comeau *et al.*, 1987).

Growth of polyP organisms can take place utilizing a number of different substrates; external, such as acetate, glucose etc., or internal, such as PHB. However, in the anaerobic/aerobic situation specific to excess P removal systems, if properly designed, all the acetate is stored as PHB in the anaerobic reactor and growth will take place in the subsequent aerobic reactor utilizing the stored PHB as the only substrate source. In the aerobic reactor a fraction of the PHB is utilized to synthesize new cell material and the remainder is oxidized to provide energy for growth and for taking up and storing P as polyP volutins; the oxidation action gives rise to the observed OUR, and the P uptake to the observed decrease in P concentration, see Figs 7.12 and 7.13: In the batch tests acetate added under anaerobic

conditions was completely sequestered (as PHB). Under the subsequent aerobic conditions both the OUR and P uptake appear to be closely associated; as there is no other significant energy source this would indicate that P uptake and storage as polyP are linked to PHB utilization.

Under aerobic conditions, should the soluble P concentration become zero, PHB utilization continues but at a reduced rate. The PHB now appears to be utilized solely for growth because no soluble P is available for uptake and storage as polyP, see Fig 7.14. The P requirements for cell synthesis probably are supplied by the polyP pool.

Ammonia is used as a nitrogen source for synthesis. Should ammonia become limiting, nitrate can serve as an alternative nitrogen source, see Fig 7.15.

- (3) Endogenous mass loss: One definition for endogenous mass loss is loss of active mass when an organism population is aerated with no substrate added, for example in the aerobic digestion of mixed liquor. This definition however, can be shown to be quite inadequate. Indeed, there exists no unambiguous definition for the term endogenous mass loss. The reason for this is that no adequate mechanistic explanation for the endogenous mass loss phenomena has evolved. Finding a mechanistic explanation has troubled research workers since Monod in (1949) "... the study of bacterial 'death', i.e. of the negative phases of growth, involves distinct problems and methods". This view also is reflected in the 1976 symposium on "The Survival of Vegetative Organisms" in which it was concluded "very little is known of the death and declining phase" (Trinci and Thurston, 1976).

Herbert (1958) was the first to attempt a quantitative description of endogenous mass loss. To account for the apparent decrease in the specific yield with increase of organism retention time he proposed that active mass is lost by oxidation to generate energy for cell maintenance. He modelled the rate of the loss as proportional to the active mass. McKinney and Ooten (1969) independently proposed endogenous mass loss and formulated it in the same manner as Herbert (1958), but extended Herbert's model by proposing that, with the mass loss was associated the generation of an inert particulate fraction, which they called the endogenous residue.

Also, they formulated mathematically the oxygen requirements and the generation of the endogenous residue in terms of the active mass loss. The consequence of hypothesizing an inert endogenous residue was that it was no longer acceptable to equate the active volatile mass to the total volatile mass. The generation of endogenous residue was confirmed by McCarty and Broderick (1962), Washington and Hetling (1965), Marais and Ekama (1976) and Warner *et al.* (1983).

Pirt (1975) proposed an alternative explanation for the phenomenon. Pirt did not recognize endogenous mass loss *per se* but accounted for the apparent decrease in specific yield with increase in sludge age, as follows: The organism requires energy to maintain essential cell function, termed 'maintenance energy'. Working with *pure cultures* Pirt hypothesized that, provided an *external substrate is available* to the organism mass, a fraction is oxidized to supply the energy requirements for maintenance, a fraction is oxidized to supply the energy requirements for growth and the balance is synthesized into new cell mass. The reduced yield with increase in sludge age was accounted for by the increased fraction of the input substrate needed to be utilized for maintenance energy of the greater sludge mass. Pirt did not address the problem of the mass loss observed where no external substrate was available to the organism mass in, for example, batch digestion.

Both the Pirt and Herbert/McKinney *et al.* models lead to similar behavioural patterns provided adequate external substrate is available. If no external substrate is provided, the Herbert/McKinney *et al.* model still provides a satisfactory explanation whereas the Pirt model provides no guidance; in this respect the Herbert/McKinney *et al.* model is superior.

A further 'philosophical' difficulty with Pirt's approach is that, with adequate external substrate available for maintenance energy, the approach implies the organism has an indefinite life span, i.e. with external substrate available an organism does not die. With regard to the Herbert/McKinney *et al.* model, although not directly stated, the model implies that organism death does occur: In a batch digestion test with time the model predicts that the active organism mass decreases, eventually to a minute fraction of the original mass, as is observed (Marais and Ekama, 1976). If the organism does not die it implies that its mass must decrease correspondingly

eventually to near zero, that is, the major fraction of the metabolic material is oxidized. This does not seem a tenable explanation for one cannot conceive of the organism decreasing below some critical mass and still remaining viable. A reasonable alternative is that a fraction of the mass must die to supply energy for maintenance of the rest. The fact that inert material is generated during digestion implies that death must have occurred leaving a remnant of unbiodegradable particulate cell material.

A deficiency in the Herbert/McKinney *et al.* model became apparent when an organism mass was subject to digestion operated under a sequence of anaerobic and aerobic periods (Warner *et al.*, 1983). Following an anaerobic period, at the beginning of the aerobic period, the oxygen demand was much higher than that measured for endogenous mass loss when the anaerobic period was not imposed. However, the total oxygen demand during the aerobic period of the anaerobic/aerobic sequence equalled the total demand in the completely aerobic digestion test for the time period spanning the anaerobic and aerobic periods. These observations cannot be accounted for in terms of the Herbert/McKinney *et al.* model which does not incorporate description of organism response under anaerobic conditions.

A model to incorporate the behavioural pattern observed above, in mixed cultures, was proposed by Dold *et al.* (1980). The antecedent to this model is of interest as it will assist in understanding the extensions to the Dold *et al.* model proposed in this chapter:

Bhatla and Gaudy (1965), while investigating the BOD-time curve, pasteurized a fraction of the inoculum (to kill protozoa) and thereafter inoculated the pasteurized and unpasteurized inocula, respectively, into a glucose-based substrate. Oxygen consumption and the numbers of bacteria and protozoa were measured over the period of the test, approximately 8 days. The pasteurized culture, after a delay, showed rapid total oxygen uptake over the subsequent 24 hours and thereafter very little additional uptake. The viable bacterial numbers increased concurrently with the oxygen demand and thereafter showed a very small rate of decline. With the unpasteurized inoculum the initial total oxygen uptake also was rapid over a period of one day; thereafter it levelled off for a short period, whereupon a secondary phase of oxygen uptake developed over the next six days, almost equal in magnitude to that

during the first phase. During the first phase of oxygen uptake the bacterial numbers increased concurrently, to approach a concentration approximately equal to that with the pasteurized inoculum. However, concomitant with the second phase of oxygen demand, the bacterial numbers declined rapidly and the protozoan numbers increased. Evidently the predation of the protozoa on the bacterial population was the cause of the second phase of oxygen demand, the bacteria serving as a substrate source for growth of the protozoa so that the oxygen uptake most probably was principally to obtain energy for protozoal synthesis.

Dold *et al.* hypothesized that, in mixed cultures, predation is a significant cause of death of organisms, liberating substrate from which the remaining organisms resynthesize new cell mass. Predation, they proposed, occurs in situations where added substrate is present and where it is absent, under aerobic conditions and short periods of anaerobiosis. In the situation where no external substrate is added, e.g. in aerobic digestion, they proposed that the oxygen demand associated with endogenous mass loss arises, in fact, from the energy requirements for resynthesis of organism mass (regeneration) from the substrate liberated by predation (death). In aerobic situations where external substrate is added Dold *et al.* proposed that the "death-regeneration", and associated oxygen demand, also is present. Under anaerobic periods predation continues liberating substrate but, because external electron acceptors are not available, the substrate accumulates causing an initial high oxygen demand under aerobic conditions subsequently imposed. They did not address the problem of "maintenance energy" but from the Bhatla and Gaudy investigation this would be relatively small compared to the regeneration energy requirements and therefore could be "absorbed" with the regeneration energy requirements. They accepted the proposals of McKinney for endogenous residue generation but modified these proposals appropriately to fit into the "death-regeneration" model. This model has performed excellently in describing activated sludge systems (IAWPRC Task Group, 1987).

In applying Dold *et al.*'s (1980) model to a monoculture or an enhanced culture, or a mixed culture from which predators have been removed, it is evident that predation cannot be present. In such systems necessarily maintenance energy effects must dominate. It is hypothesized that under

the stress of no available external substrate, a fraction of the weaker organisms will die lysing cell contents, biodegradable substrate and unbiodegradable particulate and soluble residues, into the bulk liquid. The remaining live organisms oxidize the biodegradable substrate fraction to obtain energy for maintenance only, energy for regrowth not being available. That is, in a mono or enhanced culture, in effect the rate of death will be controlled by the maintenance energy requirements. With active predation however, as is normal in mixed cultures, substrate 'liberated' is in excess of maintenance energy requirements and allows regrowth.

The mechanisms for death-maintenance described above, relate to situations where no external substrate is added to the organism mass. However, it can be hypothesized that the same mechanisms also will be active even if an externally added substrate is present, for the following reasons: There is evidence that organisms have a limited life span so that, as the culture ages, death and lysis of a fraction of the organism population will occur naturally, irrespective of whether externally added substrate is present or not, (Postgate, 1976, Trinci and Thurston, 1976). (For example, scar formation on the cell wall each time it divides, after many divisions, may weaken the organism to the extent that it splits open, Postgate, 1976). In consequence death-maintenance is hypothesized always to be present, under aerobic and anaerobic conditions, with or without substrate addition.

The death-maintenance hypothesis outlined above can be applied to the polyP organism enhanced cultures as follows:

Under *aerobic* conditions a death rate is continuously present resulting in lysis of biodegradable and unbiodegradable material to the bulk liquid. The polyP organisms, having the Entner-Duodoroff pathway (Juni, 1978), can abstract sufficient energy for maintenance functions by oxidizing the lysed biodegradable substrate. Thus the OUR under endogenous conditions (i.e. no added substrate) will be proportional to the maintenance energy requirements. The unbiodegradable lysed material cannot be utilized by the polyP organisms and appears as a particulate and soluble residue. Under the death concept the stored polyP content of the cell mass that is lost also is released to the surrounding liquid and hence the P release would be linked directly to the fraction of P per unit organism mass, and the death rate.

The explanation above is consistent with the observed behaviour. In the aerobic digestion batch tests;

- (i) the OUR is proportional to the active mass remaining indicated by the linearity of the semilog plot of OUR versus time, Fig 7.3,
- (ii) the integral of the OUR with time is linear with respect to the P concentration with time, Fig 7.5,
- (iii) the increase in P concentration is consistent with the polyP content of the lysed active mass, (this effect is shown in Chapter 8), and
- (iv) there is a build-up of soluble *apparently* unbiodegradable COD, Fig 7.6(c).

Under anaerobic conditions it can be hypothesized that death of the weaker organisms continues as described above for aerobic conditions. However, because the Entner-Duodoroff pathway cannot operate if oxygen is absent, maintenance energy requirements must be obtained by some other mechanism. We hypothesize that this energy is obtained by cleavage of stored polyP so that the P release in a digestion batch test under anaerobic conditions should be higher than under aerobic conditions. This is indeed so as indicated by comparing the P release rates in Fig 7.2(b) and Fig 7.12. The substrate released by death which, under aerobic conditions, was oxidized for maintenance energy cannot be utilized under anaerobic conditions. *For the purposes of modelling*, it is hypothesized that this substrate fraction accumulates as a soluble unbiodegradable material. This introduces an inconsistency in the model because the excess biodegradable material generated in the anaerobic phase could be utilized for growth in the aerobic phase. However, accommodation of this effect will greatly complicate the model because it implies polyP organism growth from extracellular substrate without polyP accumulation; as the effect seems to be relatively minor and was not observable experimentally, the stated hypothesized behaviour is accepted.

6. CONCLUSIONS

In this chapter a mechanistic model describing the biological excess P removal

phenomena has been presented. The model describes specifically the behaviour of polyP organisms in enhanced culture systems receiving acetate only as substrate. The behaviour indicated by this model is qualitatively in agreement with the biochemical model (Chapter 5) and the experimental observations.

This mechanistic model forms the basis for a quantitative mathematical model, to be described in Chapter 8.

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CHAPTER 8

ENHANCED POLYP ORGANISM CULTURES – KINETIC MODEL

1. INTRODUCTION

In Chapter 6 procedures have been set out whereby enhanced cultures of polyphosphate (polyP) organisms can be developed in the modified Bardenpho and UCT systems. In Chapter 7, using experimental observations made on these enhanced culture systems and on batch tests in which mixed liquors drawn from the systems were subject to a variety of conditions, a conceptual mechanistic model has been proposed to describe the biological excess P removal phenomena.

In this Chapter, using the mechanistic model as a basis, a mathematical model is developed that describes the stoichiometry and kinetics of the biological excess P removal phenomena.

2. MODEL DEVELOPMENT

The mechanistic model deals specifically with enhanced polyP organism cultures receiving acetate only as substrate. Furthermore, the model hypothesizes behaviour only under anaerobic and aerobic states. The anoxic state has not been included because, for reasons not yet understood, the enhanced culture systems have shown minimal denitrification propensity. As a consequence, the biological excess P removal processes and compounds that can be hypothesized to be involved in the anoxic state cannot be tested experimentally. It was thought preferable therefore, to omit these processes from the mechanistic model and hence also from the mathematical model. In due course, when information on the anoxic processes becomes available, these can be incorporated.

The mechanistic model identifies 11 compounds associated with 12 processes as being directly involved in biological excess P removal in enhanced culture systems. However, in these enhanced culture P removal systems, nitrification inevitably acts and it is not possible to calibrate the model without taking due cognizance of it. Consequently nitrification is included. To describe nitrification, 2 extra compounds and 2 extra processes are needed.

In developing the mathematical model for enhanced culture behaviour the final objective for modelling biological excess P removal must be kept in mind; this is, to extend the model to the mixed cultures usually encountered in nutrient removal

systems treating municipal wastewater. Clearly, there will be interactions between the polyP and non-polyP organism populations in such systems. For example:

- (1) Acetate and other short chain fatty acids must be generated by the non-polyP organisms for sequestration by the polyP organisms (Chapter 3).
- (2) Certain conceptual problems exist in regard to the death-regeneration behaviour of polyP and non-polyP organisms.

With regard to death-regeneration, there is substantial evidence that indicates polyP organisms are not predated in mixed cultures, or only insignificantly so, and suffer endogenous mass loss only for maintenance:

- (1) In mixed cultures it was shown that P release and uptake observed at different sludge ages could be explained only if the endogenous mass loss rate for polyP organisms is far lower than that for non-polyP organisms, see Chapter 3.
- (2) In enhanced polyP organism cultures, although ample opportunity exists for predator growth, this does not happen as is evident from the high specificity of the organism mass and the low endogenous mass loss rates, see Chapter 7.

Thus, in the mixed culture systems, the polyP and the non-polyP organism populations appear to behave relatively independently with only two significant links between the two populations:

- (1) Conversion of readily biodegradable COD to short chain fatty acids by the non-polyP organisms for sequestration by the polyP organisms. The kinetics of conversion have been described in Chapter 3 where it was indicated that the conversion rate is independent of the polyP organism mass; hence this link is one-directional. In anticipation of the model being developed further to apply to situations where sewage and not acetate serves as substrate, this process is included, with one extra compound.
- (2) Apparently unbiodegradable soluble COD is generated by the polyP organisms during endogenous mass loss in the enhanced culture systems. However, in mixed cultures, this apparently unbiodegradable material very likely serves as a substrate source for the non-polyP organisms. This process

of assimilation concerns the non-polyP organisms and only need be incorporated in the non-polyP kinetic model - again it is an unidirectional link.

Taking the above into account, a total of 14 compounds associated with 15 processes are identified for inclusion in the mathematical model.

3. MODEL PRESENTATION

In the mathematical model, the process rates are formulated mathematically as are the stoichiometric relationships between the processes and compounds. The large number of complex interactions between compounds and processes necessitates that these be clearly presented. Following the proposals of the IAWPRC Task Group (1987) on "Mathematical Modelling of Wastewater Treatment", the matrix format is used to present the model. This matrix format facilitates clear and unambiguous presentation of the processes and compounds of the model and their interaction. The setting up of such a matrix, how to interpret it and how it is incorporated in the mathematical solution procedure is described briefly in Appendix E. (For detailed solution procedures see Billing, 1987).

4. MODEL DESCRIPTION

The matrix for the mathematical model¹ is set out in Table 8.1. The compounds, "i" in number, are listed across the top of the matrix; the processes, "j" in number are listed down the left hand side of the matrix. Following the IAWPRC recommendation, VSS is expressed in COD units; yields and all other relevant constants are similarly expressed, see Appendix E.

To facilitate discussion the processes described in the matrix can be grouped into five categories; 4.1 conversion of "complex" readily biodegradable COD to acetate ($j = 12$), 4.2 sequestration of acetate ($j = 13$), 4.3 growth ($j = 1-4$), 4.4 endogenous mass loss ($j = 5-11$) and 4.5 nitrifier growth and decay ($j = 14-15$).

4.1 Conversion of "complex" readily biodegradable COD to acetate ($j = 12$)

This process is included for completeness. It does not operate when only acetate, or other short chain fatty acids, serve as influent, as in the enhanced culture systems. This process will be implicated in the polyP organism growth in mixed culture systems when the influent contains readily biodegradable COD other than short

¹Details of the symbol system used in the modelling are given in Appendix A.

Table 8.1: Process kinetics and stoichiometry for biological excess P removal.

COMPOUND	1	2	3	4	5	6	7	8	9	10	11	12	13	14	PROCESS RATE, ρ_j ML ⁻³ T ⁻¹
PROCESS	$Z_{B,A}$	$Z_{B,C}$	Z_E	S_{emm}	S_{phb}	P_{polyp}	$S_{bs,c}$	$S_{bs,a}$	N_{H_3}	$N_{O,bs}$	N_{O_3}	P_s	S_{us}	0	
1 Aerobic growth of $Z_{B,C}$ on S_{phb} with NH_3	1				$-\frac{1}{Y_G}$	$\frac{f_{P,upt}}{Y_G}$			$-f_{ZBG,N}$			$*A$		$-\frac{1-Y_G}{Y_G}$	$\rho_{01} \left(\frac{S_{phb}/Z_{B,C}}{K_{s01} + S_{phb}/Z_{B,C}} \right) \left(\frac{Air}{on} \right) \left(\frac{NH_3}{limit} \right) \left(\frac{P}{limit} \right) Z_{B,C}$
2 Aerobic growth of $Z_{B,C}$ on S_{phb} with NO_3	1				$-\frac{1}{Y_G}$	$\frac{f_{P,upt}}{Y_G}$					$-f_{ZBG,N}$	$*B$		$-\frac{1-Y_G}{Y_G}$	$\rho_{01} \left(\frac{S_{phb}/Z_{B,C}}{K_{s01} + S_{phb}/Z_{B,C}} \right) \left(\frac{Air}{on} \right) \left(\frac{NH_3}{limit} \right) \left(\frac{P}{limit} \right) Z_{B,C}$
3 J_1 if P_s limited	1				$-\frac{1}{Y_G}$	$-f_{ZBG,P}$			$-f_{ZBG,N}$					$-\frac{1-Y_G}{Y_G}$	$\rho_{02} \left(\frac{S_{phb}/Z_{B,C}}{K_{s02} + S_{phb}/Z_{B,C}} \right) \left(\frac{Air}{on} \right) \left(\frac{NH_3}{limit} \right) \left(\frac{1-P}{limit} \right) Z_{B,C}$
4 J_2 if P_s limited	1				$-\frac{1}{Y_G}$	$-f_{ZBG,P}$					$-f_{ZBG,N}$			$-\frac{1-Y_G}{Y_G}$	$\rho_{02} \left(\frac{S_{phb}/Z_{B,C}}{K_{s02} + S_{phb}/Z_{B,C}} \right) \left(\frac{Air}{on} \right) \left(\frac{NH_3}{limit} \right) \left(\frac{1-P}{limit} \right) Z_{B,C}$
5 Aerobic decay of $Z_{B,C}$	-1		$f_{Ep,C}$						$*C$	$*D$		$*E$	f_{EqG}	$*F$	$\rho_{03} Z_{B,C} \text{ (Air on)}$
6 Lysis of P_{polyp} for J_5						-1						1			$\rho_5 (P_{polyp}/Z_{B,C})$
7 Lysis of S_{phb} for J_5					-1			1							$\rho_5 (S_{phb}/Z_{B,C})$
8 Anaerobic decay of $Z_{B,C}$	-1		$f_{Ep,C}$						$*G$	$*H$		$*I$	$(1-f_{Ep,C})$		$\rho_8 Z_{B,C} \text{ (Air off)}$
9 Lysis of P_{polyp} for J_8						-1						1			$\rho_8 (P_{polyp}/Z_{B,C})$
10 Lysis of S_{phb} for J_8					-1			1							$\rho_8 (S_{phb}/Z_{B,C})$
11 Anaerobic cleavage of P_{polyp} for maintenance						-1						1			$\rho_{pp} P_{polyp} \text{ (Air off)}$
12 Conversion $S_{bs,c}$ to $S_{bs,a}$							-1	1							$K_C S_{bs,c} Z_{B,H} \text{ (Air off)} (1-NO_3 \text{ limit})$
13 Sequestration of $S_{bs,a}$					1	$-f_{P,rel}$		-1				$f_{P,rel}$			$K_P Z_{B,C} \text{ (Ac limit)} (PolyP \text{ limit})$
14 Aerobic growth of autotrophs	1								$-f_{ZBA,N}$ $-1/Y_A$		$\frac{1}{Y_A}$	$-f_{ZBA,P}$		$*J$	$\rho_A \left(\frac{K_{H_2}}{K_{H_2} + S_{H_2}} \right) \left(\frac{Air on}{NH_3 \text{ limit}} \right) Z_{B,A}$
15 Decay of Autotrophs	-1		$f_{Ep,A}$	$1-f_{Ep,A}$					$*K$		$*L$				$\rho_A Z_{B,A}$

$*A = -f_{ZBG,P} - f_{P,upt}/Y_G$	$*B = -f_{ZBG,P} - f_{P,upt}/Y_G$	$*C = f_{ZBG,N} - f_{P,C} f_{ZEG,N} - f_{Ea,C} f_{EaG,N}$	$*D = f_{Ea,C} f_{EaG,N}$	$*E = f_{ZBG,P} - f_{P,C} f_{ZEG,P}$	$*F = -(1-f_{Ep,C}) - f_{Ea,C}$	$*G = f_{ZBG,N} - (f_{Ep,C} f_{ZEG,N} + f_{Ea,C} f_{EaG,N})$	$*H = f_{Ea,C} f_{EaG,N}$
$*I = f_{ZBA,P} (1-f_{Ep,C})$	$*J = \frac{4.57 - Y_A}{Y_A}$	$*K = f_{ZBA,N} - f_{Ep,A} f_{ZEA,N}$	$*L = f_{ZBA,P} - f_{Ep,A} f_{ZEA,P}$				

chain fatty acids. It functions, therefore, as a link when incorporating polyP organism behaviour into the general activated sludge model. Development of the stoichiometry and kinetics of this process is as described in Chapter 3.

4.2 Sequestration of acetate ($j = 13$)

The compound acetate ($i = 8$) is taken up and stored as PHB. The rate of acetate uptake is modelled as being zero order with respect to the acetate concentration ($i = 8$) and first order with respect to the polyP organism concentration ($i = 2$), as observed in Chapter 7. The specific rate constant for sequestration is K_p . Every acetate COD that is taken up appears as PHB ($i = 5$). Concomitantly, stored polyP ($i = 6$) is cleaved and P released to the bulk liquid ($i = 12$). The polyP cleavage and P release is modelled as being directly proportional to the acetate uptake, the stoichiometric constant of proportionality being $f_{P,rel}$. Should either the acetate concentration ($i = 8$), or the stored polyP ($i = 6$) become limiting, the sequestration process terminates. This is achieved by using the switching functions (Ac limit and PolyP limit) respectively. The sequestration reaction is modelled to occur only in the absence of oxygen, in accordance with the arguments presented in Chapter 7. This is achieved by using the switching function (Air off). The sequestration process is applicable to both the first and second phase P release described in Chapter 7, only the kinetic and stoichiometric constants, K_p and $f_{P,rel}$, differ in the two phases. To model the data assembled in the batch tests, a function (P Switch) is utilized to change the constants from the first phase values to the second phase values. However, in 'normal' application of the model the first phase only is expected to be operative.

4.3 Growth ($j = 1-4$)

In line with the experimental observations in Chapter 7, modelling of aerobic growth needs to consider whether soluble P is limiting or not, and whether ammonia or nitrate is used as a nitrogen source for cell synthesis. All possible combinations of these give rise to four processes for growth, as shown in Table 8.1. However modelling of these processes has a number of common factors. Following the proposals in Chapter 7, PHB ($i = 5$) serves as the only substrate source for polyP organism growth. Being an internally stored polymer, the specific rate of utilization is modelled as a surface saturation type reaction, similar to that proposed for the rate of utilization of particulate material in the general activated sludge model (Dold *et al.*, 1980) with maximum specific growth rate $\hat{\mu}_{G_1}$ and half saturation coefficient K_{sG_1} . This function has the same basic structure as the Monod function with this exception; because the substrate is stored the rate of reaction is not proportional to

the "free" substrate concentration, but rather proportional to the substrate concentration per unit organism mass. Utilization of PHB ($i = 5$) increases the polyP organism mass ($i = 2$). If sufficient soluble P ($i = 12$) is available, a fraction of the PHB is oxidized to provide energy for growth and for taking up P ($i = 12$) and storing it as polyP ($i = 6$). P ($i = 12$) also is taken up for cell synthesis ($j = 1$ and 2). The P uptake is modelled as being proportional to the PHB utilization, in accordance with the mechanistic model in Chapter 7, the constants of proportionality being $f_{P,upt}$ for P uptake and for polyP storage, and $f_{ZBG,P}$ for cell synthesis. The oxidation of PHB ($i = 5$) causes a decrease in the oxygen concentration ($i = 14$).

Should soluble P become limiting, P uptake for polyP ($i = 8$) formation ceases, but growth continues with the P requirements for cell synthesis supplied from the polyP pool ($j = 1$ and 4). Growth under conditions where soluble P is limiting has different kinetic rate constants, ($\hat{\mu}_{G_2}$ and K_{sG_2}), than when P is not limiting. The change from one growth process to the other is achieved by the switching functions (P limit) and (1-P limit).

Ammonia ($i = 9$) is used as a nitrogen source for synthesis ($j = 1$ and 3). Should ammonia become limiting, nitrate ($i = 11$) can serve as an alternative nitrogen source for synthesis ($j = 2$ and 4), as observed in Chapter 7. This is accomplished by incorporating switching functions, (NH_3 limit) and ($1-NH_3$ limit), dependent on the ammonia concentration. A further switching function (Air on) ensures that the growth processes ($j = 1-4$) only operate if oxygen is present.

4.4 Endogenous mass loss ($j = 5-11$)

In the mechanistic model set out in Chapter 7, to account for endogenous mass two situations were identified:

- (1) When the predator action is significant, such as in mixed cultures, death produces lysed material in excess of that required for maintenance and this excess is used for regeneration of active mass. In this situation, for the purposes of modelling, the death-maintenance-regeneration is reduced to a death-regeneration approach, i.e. the maintenance energy requirement is "merged" with the energy for regeneration, because maintenance energy is a relatively small fraction of the energy requirement for regeneration.
- (2) When predator action is absent or insignificant, such as in pure cultures, death produces just sufficient lysed material for satisfying the maintenance

energy requirement, i.e. the rate of death is controlled by the maintenance energy requirement. Because the maintenance energy requirement is proportional to the active mass, the endogenous mass loss can be modelled without passing through a death-maintenance cycle. One may simply allocate a fraction of the endogenous mass loss to the oxygen demand, to account for maintenance energy requirements, and allocate the balance to some form(s) of inert material(s), the total being equal to the "maintenance death mass loss rate" for the organisms. Death now is the fractional reduction in the active mass. This is equivalent to the endogenous mass loss behaviour as conceived by McKinney and Ooten (1969).

Approach (2) is adopted to deal with endogenous mass loss for the polyP organism enhanced cultures with the difference that not only is a particulate inert fraction generated (as in McKinney and Ooten) but also an apparently inert soluble fraction insofar as the polyP organisms are concerned. (In a mixed culture this apparently inert soluble material probably will serve as a substrate source for the non-polyP organisms).

The endogenous mass loss is modelled separately for the aerobic ($j = 5-7$) and anaerobic ($j = 8-11$) states. In the aerobic state the polyP organism mass ($i = 2$) decreases ($j = 5$) generating a particulate endogenous residue ($i = 3$) and a soluble "unbiodegradable" COD ($i = 13$), that is, unbiodegradable with respect to the polyP organisms. The balance is oxidized to provide energy for cell maintenance, decreasing the oxygen ($i = 14$). The rate of mass loss is first order with respect to the polyP organism mass ($i = 2$). The ammonia ($i = 9$) increases by the difference between the amount released from endogenous mass loss, and the amount associated with the two unbiodegradable fractions generated. The stored polyP ($i = 6$) content of the mass lost is released to the bulk liquid ($j = 6$), so also the stored PHB ($j = 7$), see Chapter 7 as to the reasons for this.

In the anaerobic state the polyP organism mass ($i = 2$) also decreases ($j = 8$) generating particulate endogenous residue ($i = 3$) and soluble "unbiodegradable" COD ($i = 13$). However, as no electron acceptor (oxygen) is available, energy generation from oxidation of substrate no longer is possible and the balance of the mass loss is accumulated as soluble unbiodegradable COD ($i = 13$), that is, "unbiodegradable" with respect to the polyP organisms, see Chapter 7 for a detailed discussion. PolyP is cleaved ($j = 11$) to supply maintenance energy requirements - cleavage is modelled as being first order with respect to the polyP organism mass ($i = 2$). The ammonia

($i = 9$) increases by the difference between the amount released from endogenous mass loss, and the amount associated with the two unbiodegradable fractions generated. Furthermore, the stored polyP ($i = 6$) content of the mass lost is released to the bulk liquid as soluble P ($j = 9$), so also the stored PHB as acetate ($j = 10$).

4.5 Nitrifier growth and decay ($j = 14-15$)

This is modelled as in the general model (see Dold *et al.*, 1980, Dold and Marais, 1986).

5. TRANSPORT TERMS

The matrix presentation of the processes, compounds and the rates defines the behaviour at a single point in the system. To obtain the behaviour of any system (e.g. single completely mixed or plug flow reactor, or series of such reactors) the transport terms (e.g. mass flows and compounds into, and out of, the reactors) must be included to obtain a solution, see IAWPRC Task Group (1987). This is accomplished by setting up mass balances in time and/or space for every compound in every reactor and then solving the resultant set of equations. Details of how to set up mass balances using the matrix and solution techniques to solve the equations are described in Appendix E.

6. MODEL CALIBRATION

Calibration of the model requires that the stoichiometric and kinetic constants be quantified. These constants are listed in Tables 8.2 and 8.3 for 20°C. Essentially there are three ways whereby these constants can be quantified: (6.1) From a test in which the constant is isolated and directly measured. (6.2) From a test in which the effect of the constant is completely dominant compared to the effects of other constants. (6.3) By "curve fitting", using a range of system and batch operating conditions; this approach can be applied only if most of the other constants have been evaluated. All three methods were used to evaluate the constants.

6.1 Direct determination

The following constants were quantified by direct determination:

- (1) Specific endogenous mass loss rate (b_G): This constant was determined from the slope of the semilog plot $\log(\text{OUR})$ versus time, obtained from aerobic digestion batch tests, see Fig 7.3. The b_G values from a number of such tests are shown plotted on normal probability paper in Fig 8.1, giving a

Table 8.2: Description and values of kinetic constants in the matrix (Table 8.1).

Symbol	Constant Description	Range of Values	Units
A - POLYP ORGANISMS			
μ_{G1}	Maximum specific growth rate with no soluble P limit	0,9-1,1	/d
μ_{G2}	Maximum specific growth rate with soluble P limit	0,42	/d
K_{sG1}	Growth rate half saturation coefficient with no soluble P limit	0,18	mgCOD/l
K_{sG2}	Growth rate half saturation coefficient with soluble P limit	0,18	mgCOD/l
b_G	specific endogenous mass loss rate	0,03-0,04	/d
b_{pp}	specific polyP cleavage rate for anaerobic "maintenance" energy generation	0,03	/d
K_C	Specific rate for conversion of "complex" readily biodegradable COD to short chain acids	0,04	mgCOD/mgCOD active mass/d
K_{p1}	Specific rate of acetate uptake for first phase sequestration	6,0	mgCOD/mgCOD active mass/d
K_{p2}	Specific rate of acetate uptake for second phase sequestration	2,6	mgCOD/mgCOD active mass/d
B - AUTOTROPHS (NITRIFIERS)			
μ_A	Maximum specific growth rate	$\pm 0,35$	/d
K_{NH}	Growth rate half saturation coefficient	1,0	mgN/l
b_A	Specific decay rate	0,04	/d

Table 8.3: Description and values of stoichiometric constants in the matrix (Table 8.1).

Constant		Range of Values	Units
Symbol	Description		
A - POLYP ORGANISMS			
Y_G	Yield	0,639	mgCOD volatile mass/mgCOD
$f_{P,upt}$	Ratio P uptake/COD stored substrate utilized	0,9-1,1	mgP/mgCOD
$f_{ZBG,N}$	Nitrogen content of active mass	0,07	mgN/mgCOD active mass
$f_{ZEG,N}$	Nitrogen content of endogenous mass	0,07	mgP/mgCOD active mass
$f_{EsG,N}$	Nitrogen content of soluble unbiodegradable COD	0,07	mgN/mgCOD
$f_{ZBG,P}$	Phosphorus content of active mass (excluding polyP)	0,021	mgP/mgCOD active mass
$f_{ZEG,P}$	Phosphorus content of endogenous mass	0,021	mgP/mgCOD endogenous mass
$f_{Ep,G}$	Fraction of active mass that remains as particulate unbiodegradable residue	0,25	mgCOD endogenous mass/mgCOD active mass
$f_{Es,G}$	Fraction of active mass that remains as soluble unbiodegradable residue	0,20	mgCOD/mgCOD active mass
$f_{P,rel1}$	Ratio P release/acetate uptake for phase 1 sequestration	0,48-0,55	mgP/mgCOD
$f_{P,rel2}$	Ratio P release/acetate uptake for phase 2 sequestration	0,8-1,0	mgP/mgCOD
f_{Switch}	Changes acetate sequestration from phase 1 to phase 2, i.e. $f_{P,rel1}$ to $f_{P,rel2}$ and K_{p1} to K_{p2}	0,32	mgP/mgCOD active mass
f_{cv}	Ratio COD/VSS	1,42	mgCOD/mgVSS
B - AUTOTROPHS (NITRIFIERS)			
Y_A	Yield	0,15	mgCOD volatile mass/mgCOD
$f_{ZBA,N}$	Nitrogen content of active mass	0,068	mgN/mgCOD active mass
$f_{ZEA,N}$	Nitrogen content of endogenous mass	0,068	mgN/mgCOD endogenous mass
$f_{ZBA,P}$	Phosphorus content of active mass	0,02	mgP/mgCOD active mass
$f_{ZEA,P}$	Phosphorus content of endogenous mass	0,02	mgP/mgCOD endogenous mass
$f_{Ep,A}$	Fraction of active mass that remains as particulate unbiodegradable residue	0,08	mgCOD endogenous mass/mgCOD active mass

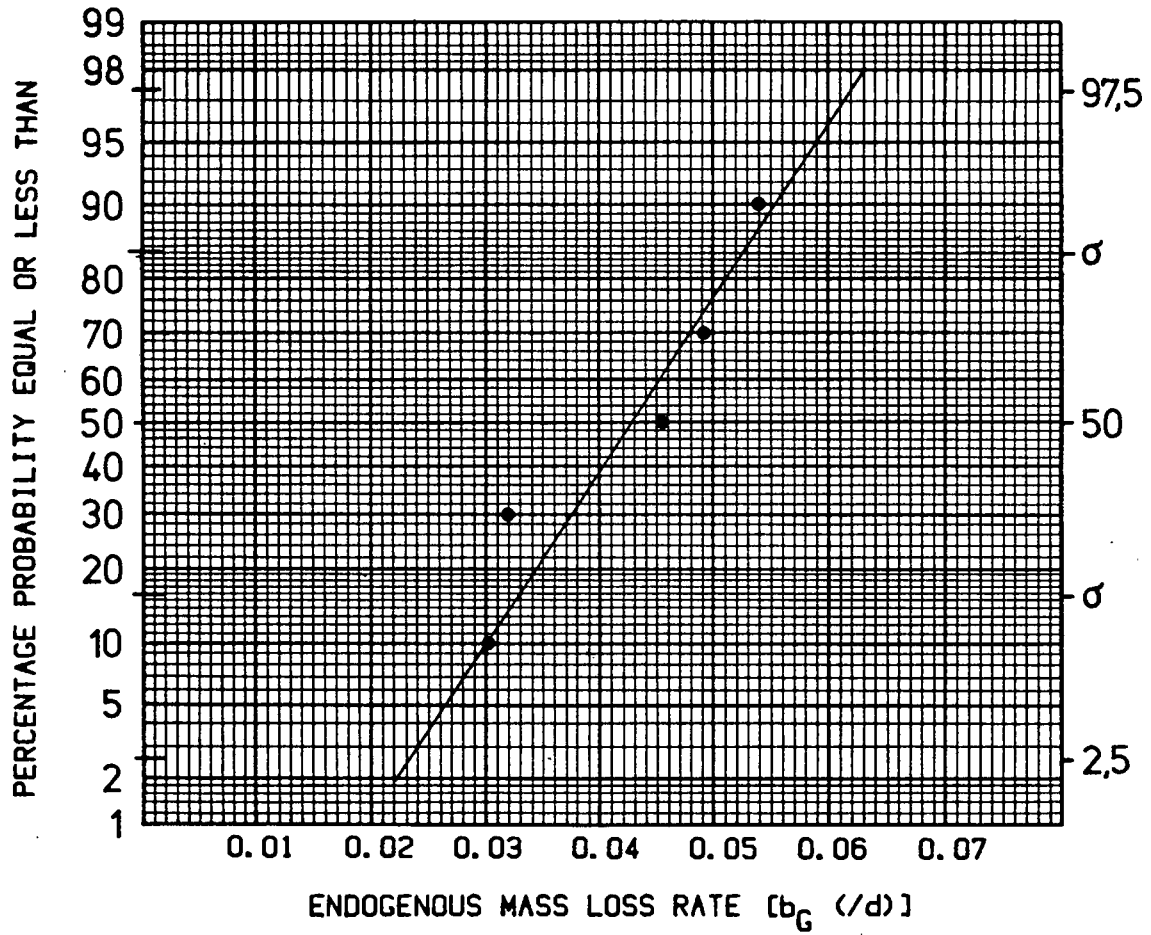


Fig 8.1: Statistical plot of endogenous mass loss rates (b_G) obtained from a number of aerobic digestion batch tests, such as in Fig 7.3.

mean value for b_G of 0,04/d with a standard deviation of the mean of $\pm 0,004/d$.

- (2) COD/VSS ratio (f_{cv}): An aliquot of mixed liquor was drawn from the last aerobic reactor of the enhanced culture systems. A sample was taken for VSS and TSS determinations. A further sample was diluted appropriately with distilled water and macerated in a high speed blender and the COD of the resultant macerated mixed liquor measured. From the two results, f_{cv} was determined. A number of f_{cv} values were determined on the enhanced culture systems over a period of two months during steady state conditions. These are shown plotted on normal probability paper in Fig 8.2, giving a mean value $f_{cv} = 1,42 \text{ mgCOD/mgVSS}$ with a standard deviation of the mean of $\pm 0,013$. This mean value conforms to the theoretical value obtained for the empirical stoichiometric formulation for biological sludge of $C_5H_7O_2N$ (WRC, 1984).
- (3) Fractional nitrogen content of active ($f_{ZBG,N}$) and endogenous ($f_{ZEG,N}$) masses: The fractional nitrogen content of active ($f_{ZBG,N}$) and endogenous ($f_{ZEG,N}$) masses are given by the TKN/COD ratio provided it is assumed that the ratio is the same for the active and endogenous masses. Furthermore, the COD/VSS ratio can be assumed to be constant and equal 1,42 mgCOD/mgVSS, as shown above. Hence, the TKN/COD ratio can be determined from TKN and VSS measurements on the mixed liquor, the latter measurement being more simple than direct measurement of COD. The TKN/VSS values from a number of such tests are shown plotted on probability paper in Fig 8.3, giving a mean value of 0,102 mgN/mgVSS with a standard deviation of the mean of 0,0013. The TKN/COD ratio then is equal to $(TKN/VSS)/1,42$, i.e. 0,072 mgN/mgCOD. The TKN/COD ratio of the soluble unbiodegradable endogenous residue ($f_{EsG,N}$) is assumed to be equal to this value.
- (4) Fractional phosphorus content of active ($f_{ZBG,P}$) and endogenous ($f_{ZEG,P}$) masses: This phosphorus content excludes the polyP. Values for these constants were obtained from the literature due to the difficulty in separating the polyP from the normal cell phosphorus content. Values in the literature range from 0,01 to 0,03 mgP/mgVSS (Mino *et al.*, 1984, WRC, 1984). The value 0,02 mgP/mgVSS is accepted.

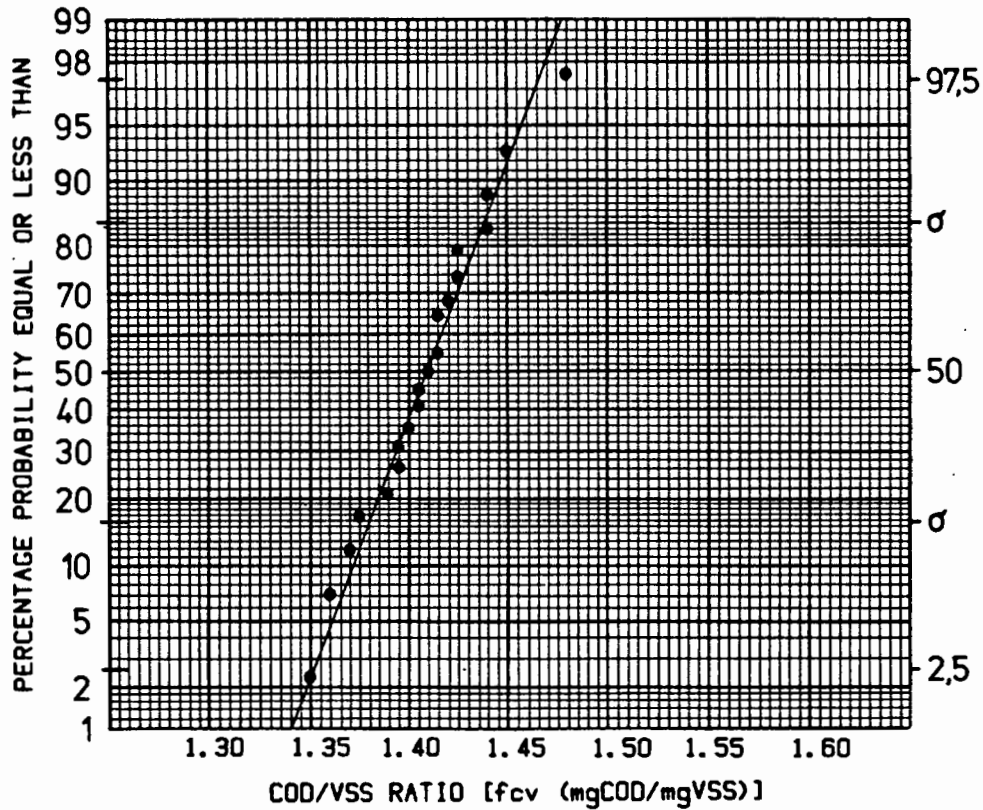


Fig 8.2: Statistical plot of COD/VSS ratios (f_{cv}) measured for mixed liquor from the polyP organism enhanced culture systems.

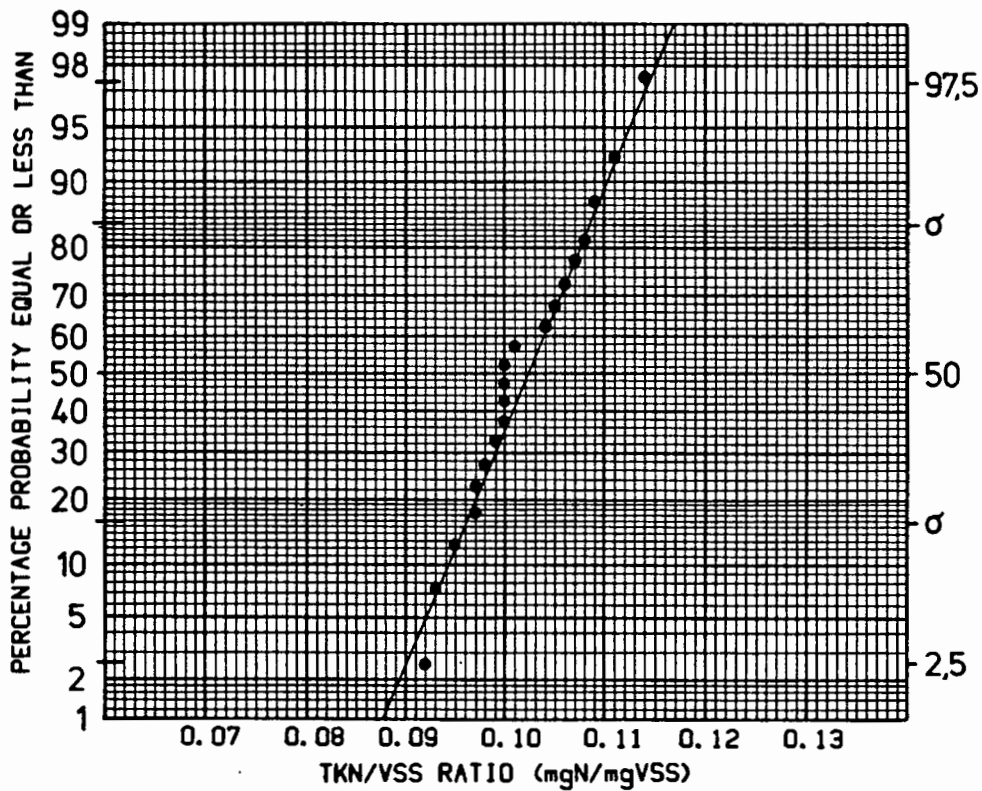


Fig 8.3: Statistical plot of TKN/VSS ratios measured for mixed liquor from the polyP organism enhanced culture systems.

6.2 Dominant behaviour

Constants obtained from dominant behaviour were the following:

- (1) Ratio P release/Acetate uptake ($f_{P,rel}$): This ratio was obtained from the anaerobic batch tests with acetate addition, see, Figs 7.7 and 7.8, for both the first (f_{P,rel_1}) and the second (f_{P,rel_2}) phases of sequestration; it is assumed that the rate of acetate sequestration in these tests is so rapid that endogenous effects can be taken as negligible. The ratios are obtained by plotting acetate concentration versus P concentration, the slope of the line defining $f_{P,rel}$. Values from a number of such plots are shown plotted on probability paper, Figs 8.4 and 8.5, for f_{P,rel_1} and f_{P,rel_2} respectively, giving mean values of $f_{P,rel_1} = 1,09$ mmolP/mmol acetate and $f_{P,rel_2} = 1,81$ mmolP/mmol acetate with standard deviations of the means as $\pm 0,038$ and $\pm 0,072$ respectively. This implies that in the second phase more P must be released to sequester one unit of acetate than in the first phase. Converting units, $f_{P,rel_1} = 0,52$ mgP/mgCOD(HAc) and $f_{P,rel_2} = 0,88$ mgP/-mgCOD(HAc).
- (2) Specific rate of acetate sequestration (K_p): This constant was obtained by linear regression analysis of the acetate increase during the same anaerobic batch tests with acetate addition as in section 6.2(1) above. Again it is assumed that the rate of acetate sequestration is so rapid that other effects can be neglected. Specific rate constants, i.e. rate per unit VSS, from a number of such tests are shown plotted on probability paper in Figs 8.6 and 8.7 for phase one (K_{p1}) and phase two (K_{p2}) sequestration respectively, giving means of $K_{p1} = 6,7$ mgHAc/mgVSS/d and $K_{p2} = 2,91$ mgHAc/mgVSS/d and standard deviations of the mean of $\pm 0,29$ and $\pm 0,02$ respectively. Recalculating, to take into account endogenous mass and to convert to COD units, $K_{p1} = 6,07$ mgCOD(HAc)/mgCOD active mass/d and $K_{p2} = 2,67$ mgCOD(HAc)/mgCOD active mass/d.
- (3) Change from first to second phase sequestration (PSwitch): The change from first to second phase sequestration was modelled to take place when the stored polyP/(polyP organism active mass) declined below the value, PSwitch. The value for PSwitch was obtained from the anaerobic batch tests described above by subtracting the mass of P released during first phase sequestration from the initial polyP concentration and dividing by the active mass of organisms. This gives a value PSwitch = 0,31 mgP/mgCOD

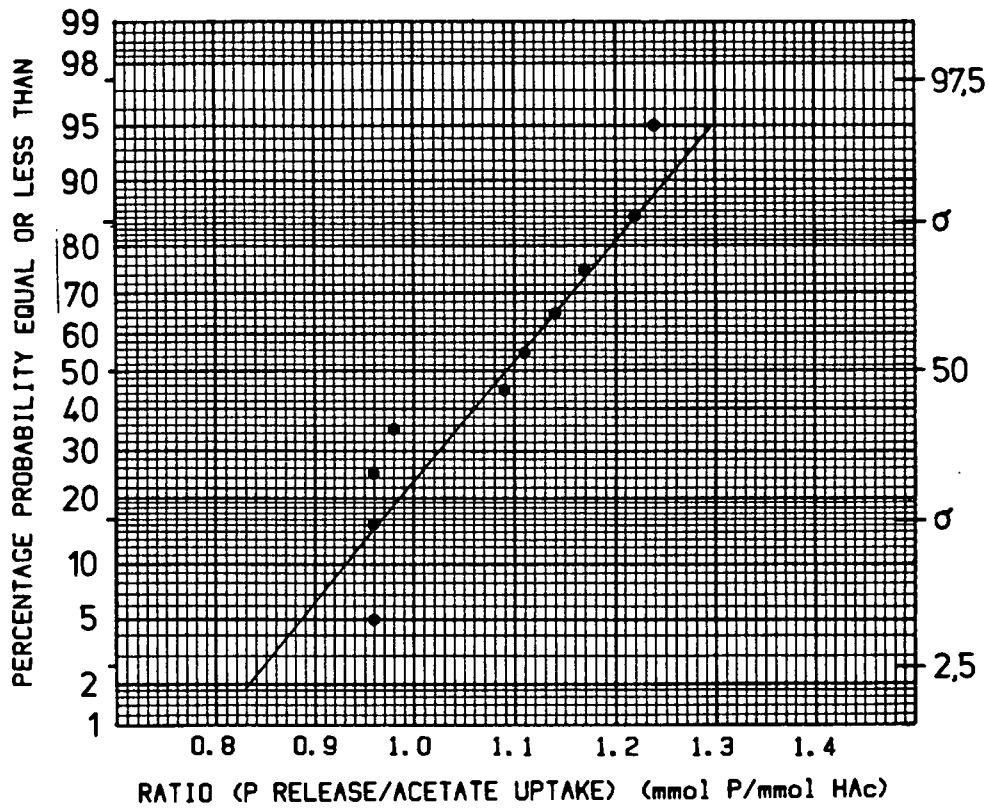


Fig 8.4: Statistical plot of first phase stoichiometric ratio between anaerobic P release and acetate uptake (f_{P,rel_1}).

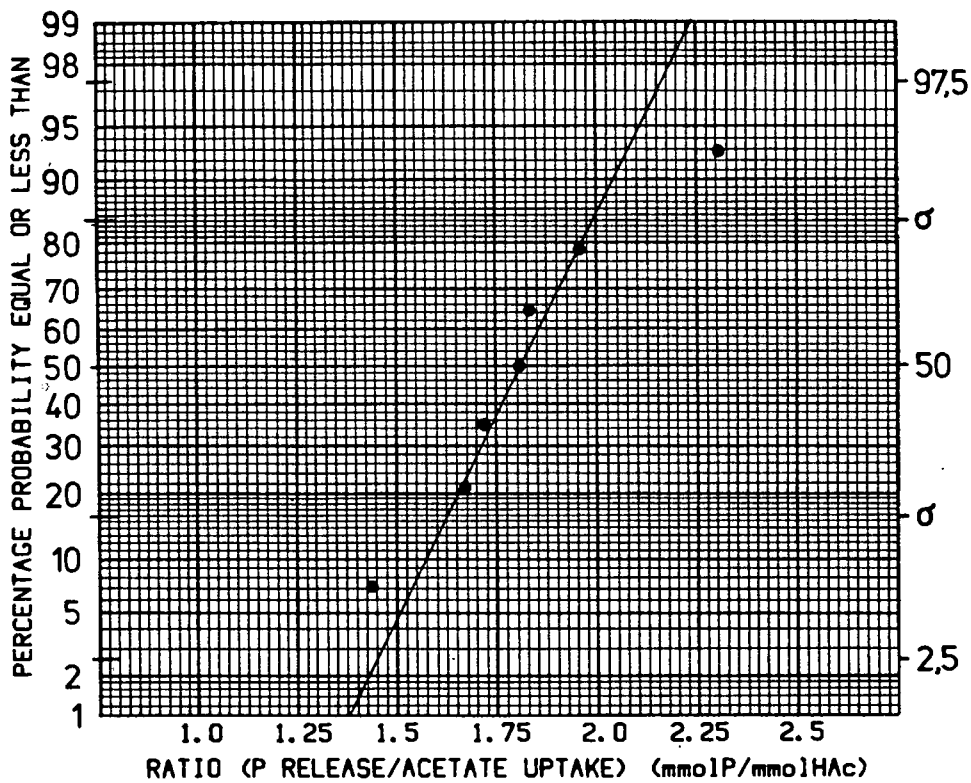


Fig 8.5: Statistical plot of second phase stoichiometric ratio between anaerobic P release and acetate uptake (f_{P,rel_2}).

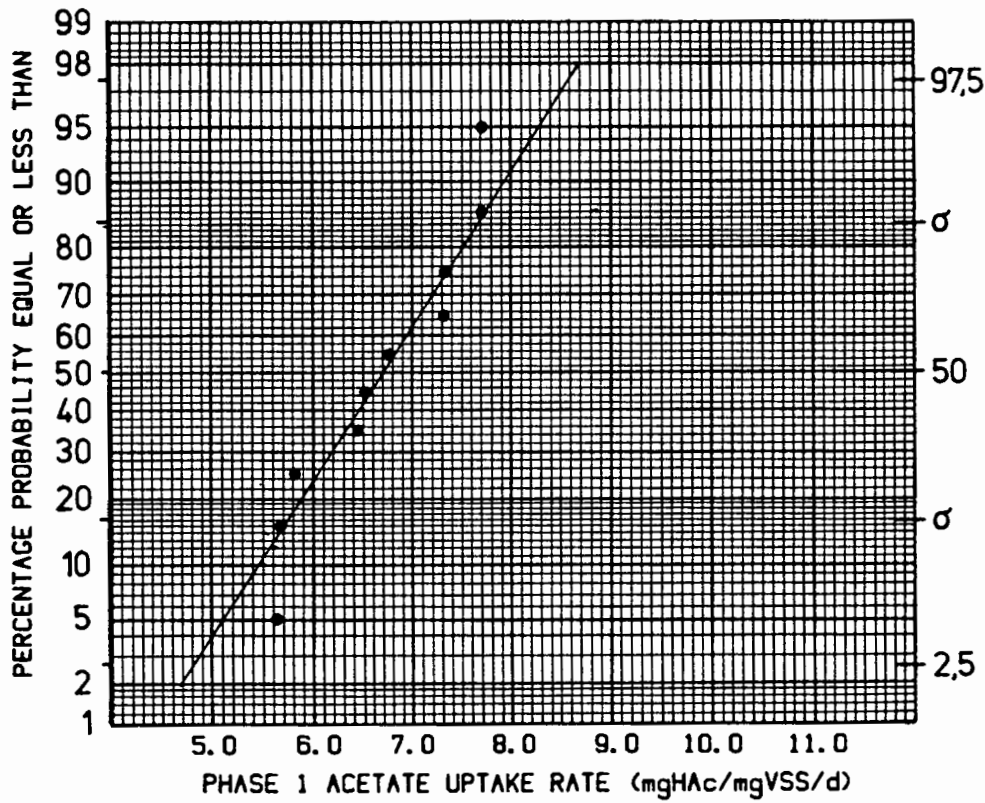


Fig 8.6: Statistical plot of first phase rates of acetate uptake (K_{p1}) in anaerobic batch tests such as in Fig 7.7.

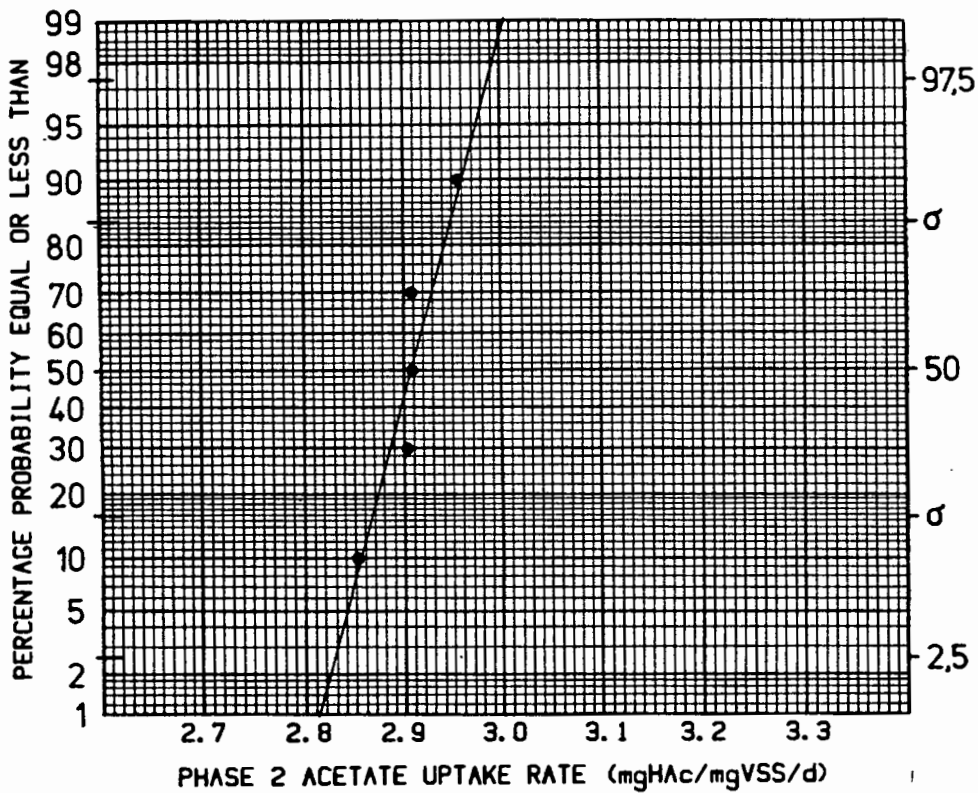


Fig 8.7: Statistical plot of second phase rates of acetate uptake (K_{p2}) in anaerobic batch tests such as in Fig 7.8.

active mass. The constant (PSwitch) comes into operation only in situations of high acetate loading, e.g. in batch tests. As described in Chapter 7, the acetate loading rates encountered in anaerobic reactors of enhanced culture systems probably will be insufficient to stimulate second phase P release.

- (4) Yield (Y_G): In Chapter 7 the difficulties associated with determining the actual specific yield were briefly described. However, where the substrate is completely biodegradable and soluble, a direct technique for determining the actual specific yield (Y_G) is possible by utilizing the data obtained from the batch tests in which the batch is aerated following anaerobic acetate addition, see Figs 7.12 and 7.13. From the matrix (Table 8.1) it is evident that a COD mass balance must be obtained in the growth process. By monitoring the OUR, the mass of oxygen consumed for COD oxidation following anaerobic addition of a known mass of acetate can be calculated by integrating the OUR–time plot and subtracting the oxygen demand for nitrification and endogenous mass loss. The value for Y_G is determined to be the value that gives equality between the mass of COD added and the mass of COD recovered. Using this technique Y_G values were obtained from a number of such tests, shown plotted on probability paper in Fig 8.8, giving a mean $Y_G = 0,639$ mgCOD active mass/mgCOD consumed and standard deviation of the mean of $\pm 0,0128$. Dividing by the f_{cv} , measured earlier, gives $Y_G = 0,45$ mgVASS/mgCOD. This conforms to the values determined for general activated sludge by Dold *et al.*, (1980).

6.3 Curve fitting

The following constants were obtained by curve fitting:

- (1) Maximum specific growth rate ($\hat{\mu}_G$) and half saturation coefficient (K_{sG}): The maximum specific growth rate ($\hat{\mu}_G$) and half saturation coefficient (K_{sG}) for PHB utilization with no soluble P limitation ($\hat{\mu}_{G_1}$ and K_{sG_1} respectively) are obtained by fitting the predicted carbonaceous OUR to the carbonaceous OUR observed in the aerobic batch tests following anaerobic acetate addition, see Figs 7.12 and 7.13. The values for $\hat{\mu}_{G_1}$ and K_{sG_1} determine the shape of the OUR curve; only one pair of data values provide predictions that fit the observed curve. Furthermore, the effect of $\hat{\mu}_G$ and K_{sG} dominate completely in these tests so that selection of values for the

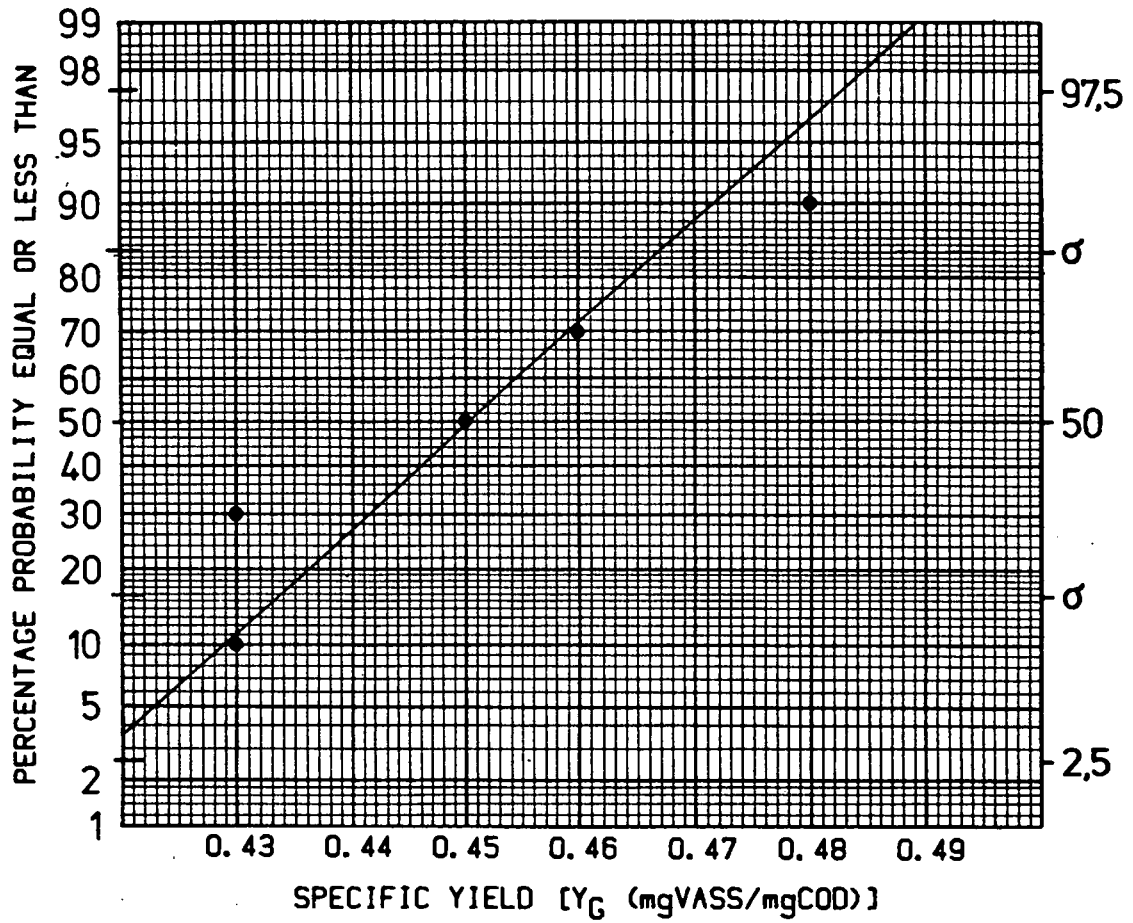


Fig 8.8: Statistical plot of yield (Y_G) values obtained from COD mass balances on aerobic batch tests following anaerobic acetate addition, such as in Fig 7.12.

constants is not influenced by other constant values. The range of values obtained for a number of such tests are listed in Table 8.3.

The maximum specific growth rate and half saturation coefficient for PHB utilization with a soluble P limitation ($\hat{\mu}_{G_2}$ and K_{sG_2} respectively) were obtained in a similar manner from an aerobic batch test (after anaerobic acetate addition) in which the soluble P reduced to zero, see Fig 7.14.

- (2) Ratio P uptake/stored COD utilization ($f_{P,upt}$): This ratio was obtained from the P concentration changes observed in the aerobic batch tests described for section 6.3(1) above. It is assumed that the P concentration changes dominate over the changes due to endogenous respiration, so that the effect due to $f_{P,upt}$ is not influenced by predetermination of other constants. The range of $f_{P,upt}$ values obtained in this manner is listed in Table 8.2.
- (3) Specific polyP cleavage rate for anaerobic "maintenance" energy generation (b_{pp}): This rate was determined by trial curve fitting to the increase in the P in the anaerobic batch tests after the acetate concentration becomes zero, see Figs 7.7 and 7.8. This requires that the endogenous mass loss rate (b_G) already is determined. Ranges of values obtained for b_{pp} are listed in Table 8.3.
- (4) Endogenous residue fractions ($f_{Ep,G}$ and $f_{Es,G}$): Both the particulate ($f_{Ep,G}$) and the soluble ($f_{Es,G}$) endogenous residue fractions were determined from the aerobic digestion batch tests, see Figs 7.2 and 7.6. The magnitude of the soluble unbiodegradable COD fraction ($f_{Es,G}$) was determined by fitting the predicted data to the soluble COD time curve observed in the batch test, see Fig 7.6(c). The particulate endogenous residue fraction ($f_{Ep,G}$) was determined by fitting the predicted OUR to that observed in the batch tests, see Fig 7.6(a). By knowing b_G and $f_{Es,G}$ only one value of $f_{Ep,G}$ will provide conformity between predicted and observed OUR. Values obtained for $f_{Ep,G}$ and $f_{Es,G}$ are listed in Table 8.2.
- (5) Switching functions: The switching functions utilized in the model are set out in Table 8.4. Values for constants were determined by "curve fitting"; very small values are selected to meet the requirement that the function reduces to zero only at very low concentrations, see Appendix E.

With regard to the nitrifiers, the yield coefficient (Y_A), fractional N contents of the endogenous residue ($f_{ZEA,N}$) and the active mass ($f_{ZBA,N}$), the half saturation coefficient for growth (K_{NH}) and the specific endogenous mass loss rate (b_A) were taken from typical values accepted in the IAWPRC model (Dold and Marais, 1986).

The nitrifier maximum specific growth rate ($\hat{\mu}_A$) was determined by "curve fitting", to the nitrification curve in aerobic batch tests after anaerobic acetate addition.

7. MODEL VERIFICATION

The acceptability of the model is promoted if, on applying it to a range of situations, one finds consistency between observation and prediction. In this respect one can distinguish two types of verification, against batch and steady state system response.

7.1 Batch verification

In Figs 8.9 to 8.15, the predicted behaviour of various compounds is compared with those observed in the different batch tests described in Chapter 7. (It should be noted that these plots are examples selected to represent the various types of batch tests; a number of each type of batch test were conducted, all of which show similar results). Note that the data in Fig 7.18 (showing the direct utilization of acetate for growth under aerobic conditions simultaneous with acetate uptake, PHB storage and utilization, and P release and uptake) is not modelled for reasons set out in Chapter 7.

Even though the same set of tests has been used for evaluating some of the constants, the closeness with which the predictions conform to the observations over the wide range of conditions in the batch tests constitutes evidence for the acceptability of the model.

7.2 Steady state system verification

In simulating the steady state systems responses it should be noted that:

- (1) The model does not incorporate denitrification; accordingly it cannot simulate the denitrification observed experimentally. A method for taking the denitrification effects into account can be developed from the following experimental observations:
 - (i) Denitrification was minimal in the anoxic zones of the systems where the acetate concentration was zero, and the stored PHB at a maximum. This would indicate that PHB is not utilized for

Table 8.4: Switching functions used in the matrix (Table 8.1)

Abbreviation	Formulation	Value of half saturation coefficient (K)
Air on	$\frac{0}{K_{OH} + 0}$	$K_{OH} = 0,002 \text{ (mgO/l)}$
Air off	$\frac{K_{OH}}{K_{OH} + 0}$	
NH ₃ limit	$\frac{N_{h3}}{K_{NA} + N_{h3}}$	$K_{NA} = 0,05 \text{ (mgN/l)}$
NO ₃ limit	$\frac{N_{o3}}{K_{NO} + N_{o3}}$	$K_{NO} = 1,0 \text{ (mgN/l)}$
P limit	$\frac{P}{K_{LP} + P}$	$K_{LP} = 0,1 \text{ (mgP/l)}$
PolyP limit	$\frac{P_{polyP}}{K_{xp} + P_{polyP}}$	$K_{XP} = 1,0 \text{ (mgP/l)}$
Ac limit	$\frac{S_{bs,a}}{K_{SSEQ} + S_{bs,a}}$	$K_{SSEQ} = 1,0 \text{ (mgCOD/l)}$

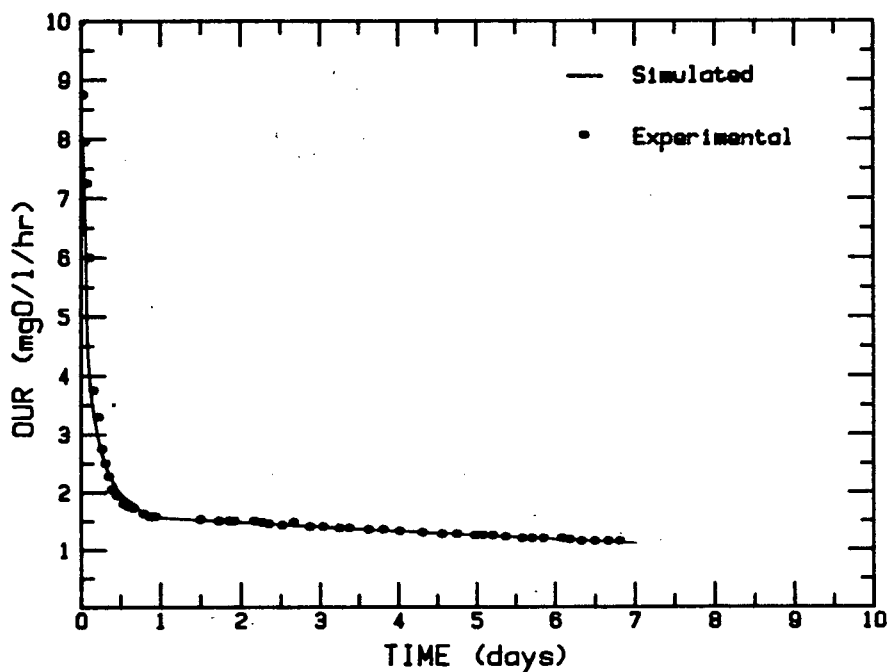


Fig 8.9(a): Experimentally observed and simulated oxygen utilization rate (OUR) response with time in a batch aerobic digestion of mixed liquor from the enhanced culture system (VSS = 1096 mgVSS/l).

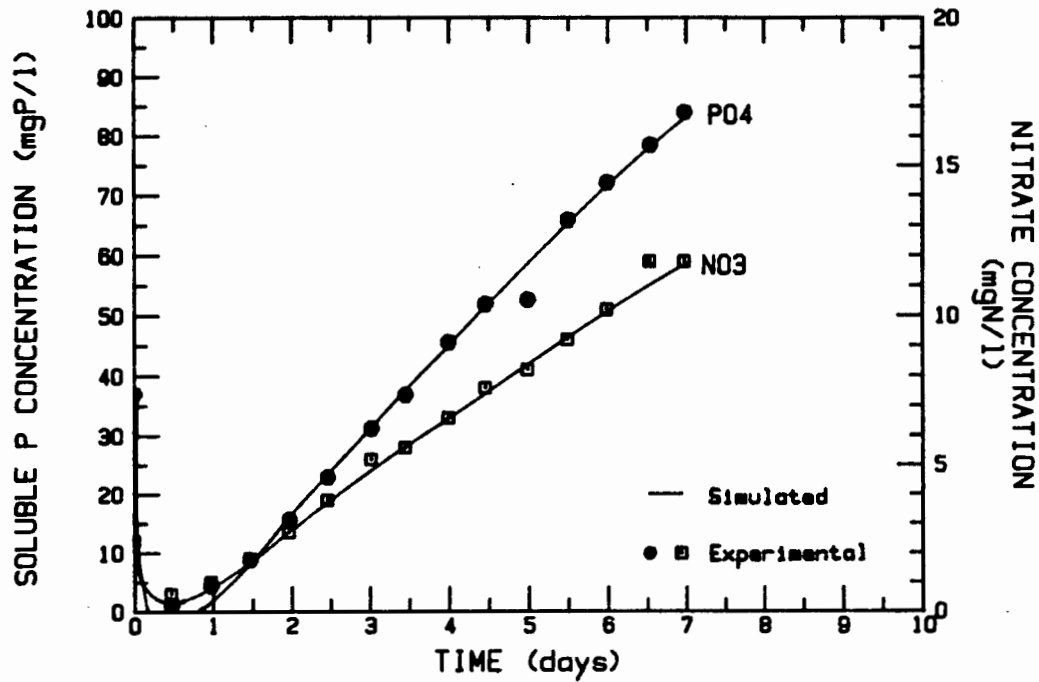


Fig 8.9(b): Experimentally observed and simulated total soluble phosphorus (PO_4) and nitrate (NO_3) concentration-time profiles for the batch aerobic digestion in Fig 8.9(a).

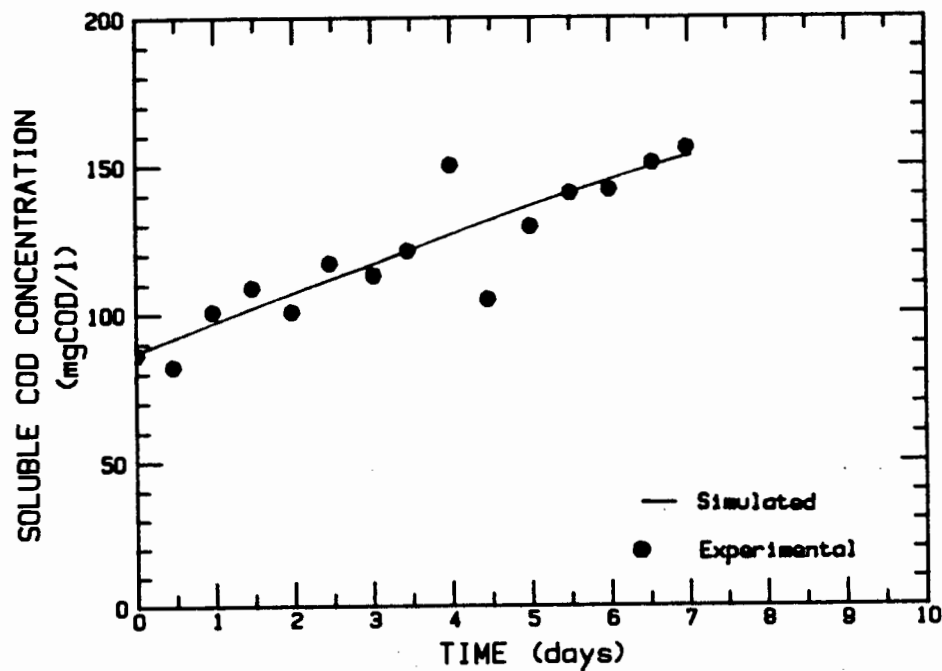


Fig 8.9(c): Experimentally observed and simulated filtered COD concentration-time profiles for the batch aerobic digestion in Fig 8.9(a).

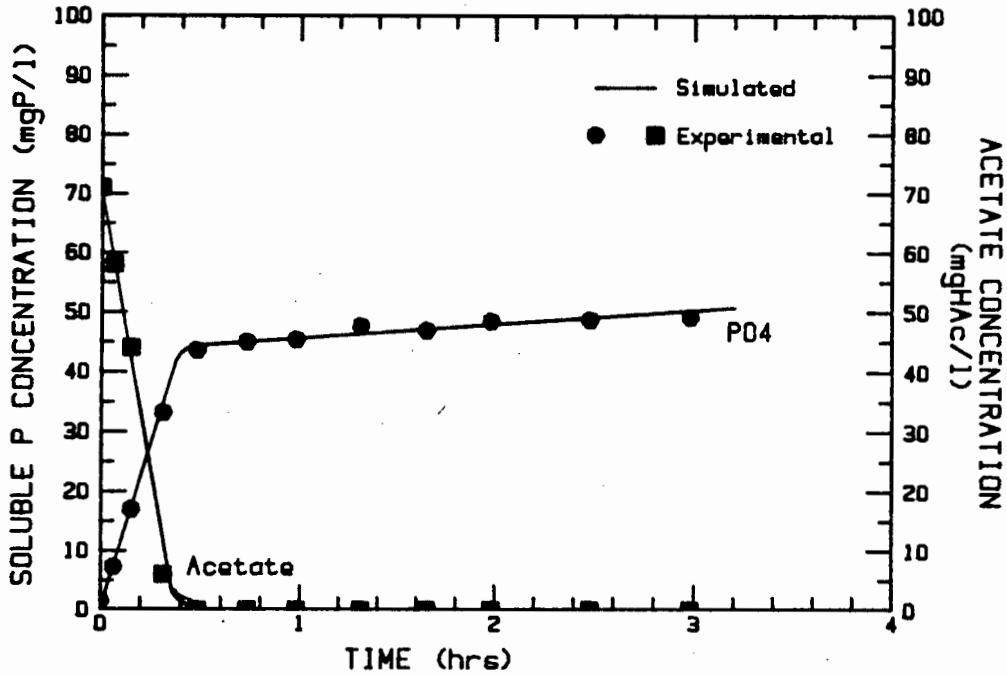


Fig 8.10: Experimentally observed and simulated total soluble phosphate (PO_4) and acetate concentration-time profiles with anaerobic addition of 0,11 mgCOD acetate/mgVSS to a mixed liquor batch drawn from the enhanced culture system ($\text{VSS} = 684 \text{ mgVSS/l}$).

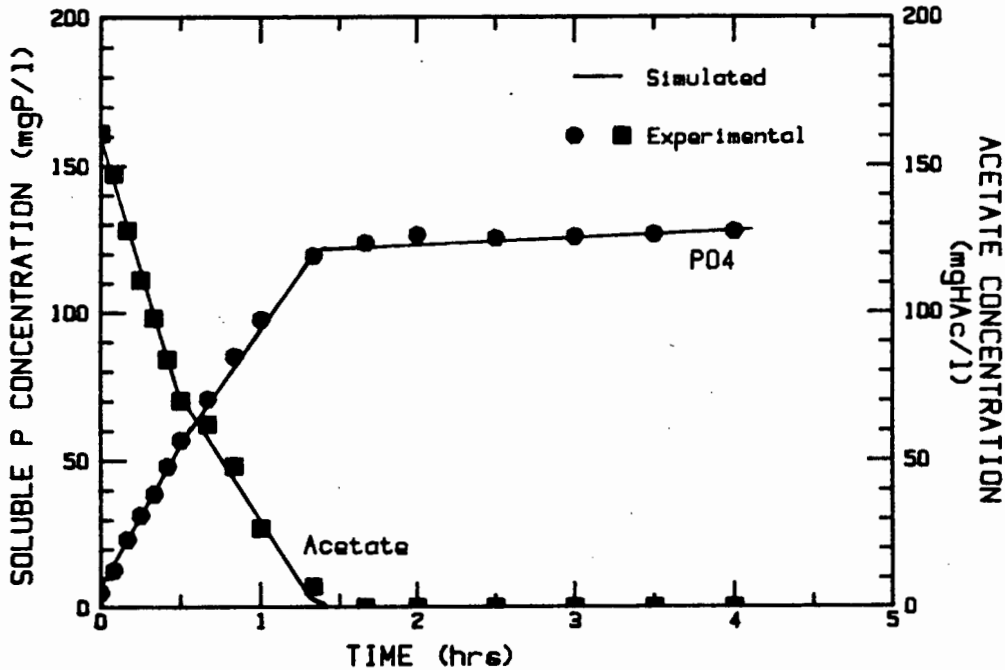


Fig 8.11: Experimentally observed and simulated total soluble phosphate (PO_4) and acetate concentration-time profiles with anaerobic addition of 0,265 mgCOD acetate/mgVSS to a mixed liquor batch drawn from the Bardenpho enhanced culture system ($\text{VSS} = 651 \text{ mgVSS/l}$).

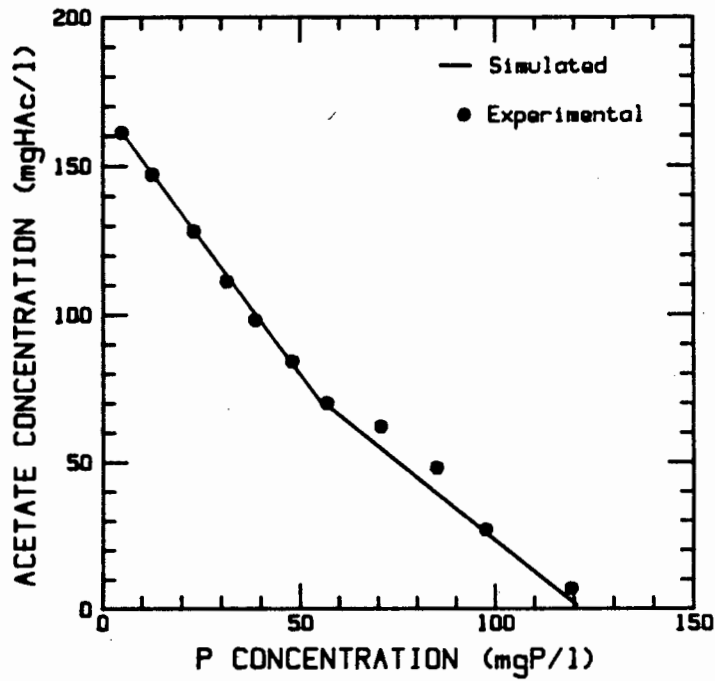


Fig 8.12: Plot of experimentally observed and simulated acetate concentration versus total soluble phosphate concentration for the time paired data in Fig 8.11.

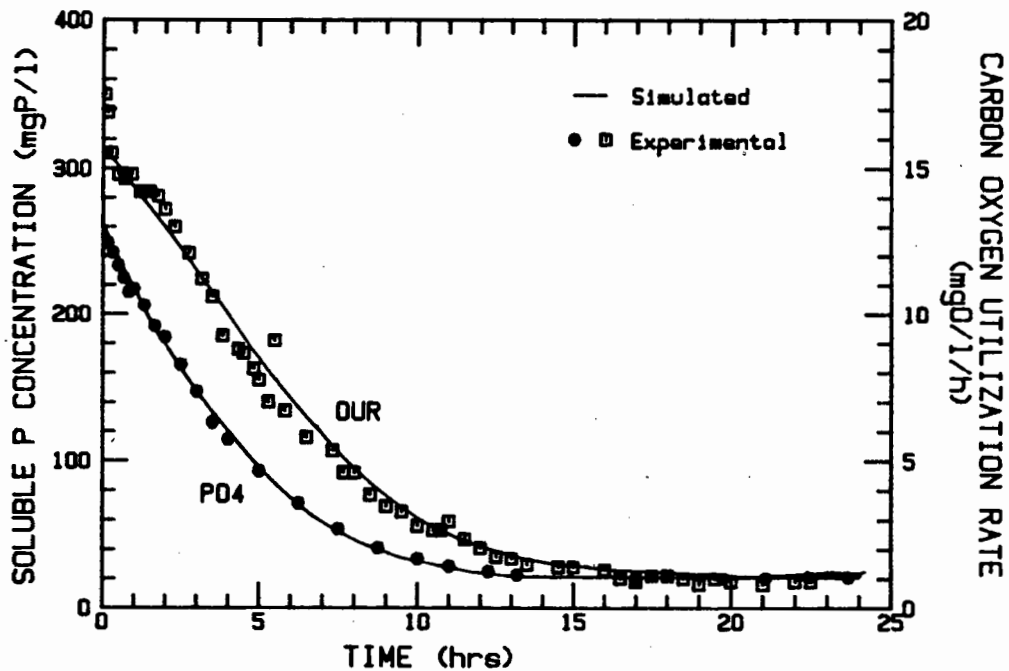


Fig 8.13: Experimentally observed and simulated total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0,207 mgCOD acetate/mgVSS to mixed liquor batch drawn from the enhanced culture system (VSS = 1041 mgVSS/l).

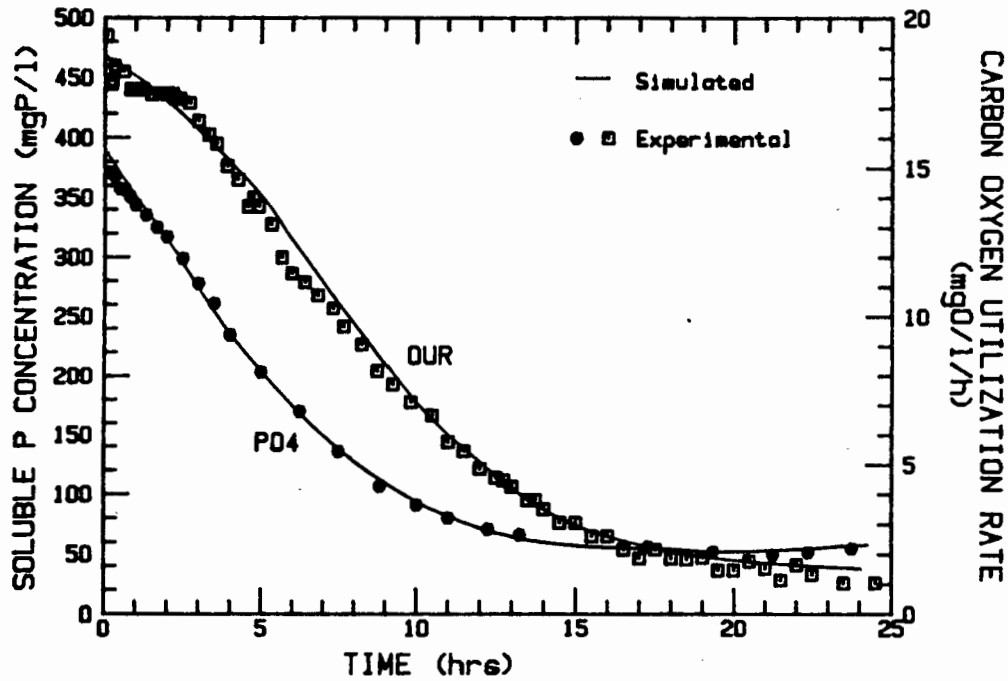


Fig 8.14: Experimentally observed and simulated total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0,363 mgCOD acetate/mgVSS to mixed liquor batch drawn from the enhanced culture system (VSS = 1199 mgVSS/l).

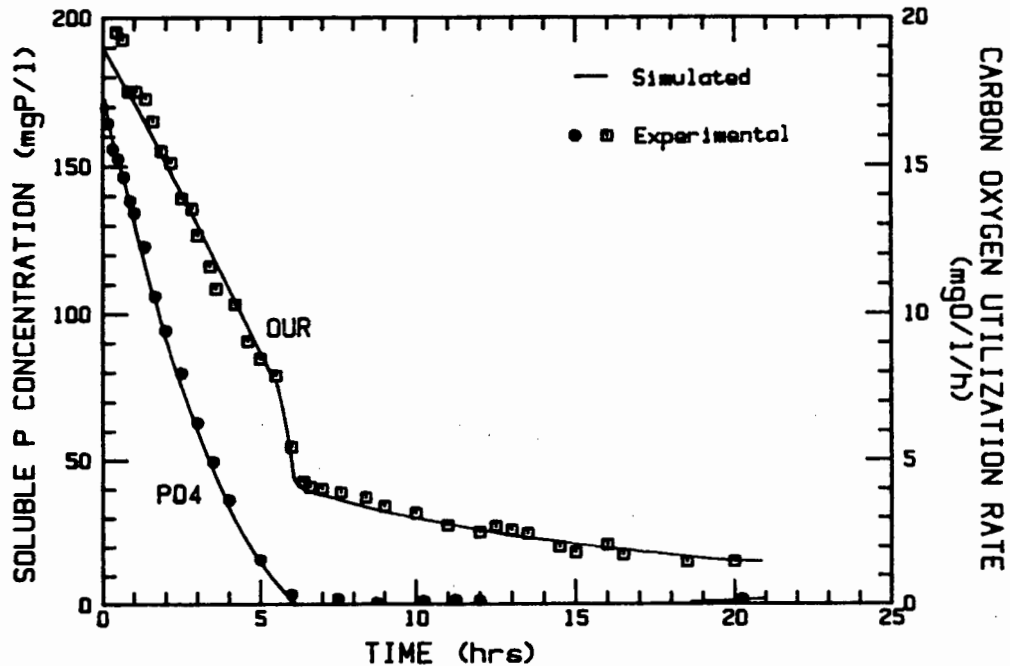


Fig 8.15: Experimentally observed and simulated total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilization rate (OUR) on aeration following anaerobic acetate addition of 0,22 mgCOD acetate/mgVSS to mixed liquor batch drawn from the enhanced culture system. The PO_4 concentration falls to zero during the course of this test (VSS = 1226 mgVSS/l).

denitrification. (This also was observed in the batch experiments, see Chapter 7, section 4.4).

- (ii) Denitrification took place almost totally in the anaerobic reactor, in the first anaerobic reactor for two anaerobic reactors in series. This would indicate that denitrification takes place virtually only while "free" acetate is present.

These two observations imply that the major fraction of the denitrification in the system is mediated by the non-polyP organisms in the anaerobic reactor with acetate as electron donor. The mass of these organisms produced by the denitrification action would be relatively small, as indicated by the high specificity of the organism population (see Chapter 7) and the relatively small fraction of the total influent COD implicated in denitrification. One can accept, therefore, that the non-polyP organism mass effect on the total volatile mass is minor and can be neglected, which is equivalent to assuming that the COD in the influent available to the polyP organisms is reduced by the COD lost in denitrification. This assumption will give rise to a slightly high prediction of the specific yield constant for the polyP organisms. In a large measure this problem will resolve itself when the polyP organism model is incorporated into the general model.

- (2) The yeast extract added to the influent is a complex organic material, and appears to be unbiodegradable with respect to the polyP organism mass in which event it will pass unaltered to the effluent. This conclusion arises from the simulation studies on the systems, in which it was noted that with higher yeast extract addition the effluent COD also appears to be raised relative to the influent COD. If the COD of the yeast extract is added to the influent substrate as unbiodegradable soluble COD, then the system effluent COD is closely predicted by the sum of the concentration of unbiodegradable soluble COD generated by endogenous mass loss (using the constants derived from the batch tests) and the COD of the yeast extract.

Taking the above two factors into account, the influent concentration *available* for polyP organism metabolism is given by:

$$\begin{aligned}\text{COD(available)} &= \text{COD(measured in influent)} \\ &\quad - \text{COD(associated with denitrification)} \\ &\quad - \text{COD(yeast extract)}.\end{aligned}$$

In the simulation, the 'predicted' influent COD is given by:

$$\begin{aligned}\text{COD('predicted' influent)} &= \text{COD(measured in influent)} \\ &\quad - \text{COD(denitrification)}.\end{aligned}$$

The COD (yeast extract) is retained in the 'predicted' influent value because the model makes provision for this COD fraction and passes it through the system as unbiodegradable soluble material.

Using the method above for determining the influent COD, in simulating the systems responses it was found that all the constant values derived from the batch tests could be kept the same, except for two - the maximum specific growth rate, $\hat{\mu}_{G_1}$ (increased from 1 to 1,2/d) and the ratio P uptake per mgCOD (as PHB) utilized, $f_{P,upt}$ (reduced from 1,0 to 0,75 mgP/mgCOD). Although it is not possible to give a determinative explanation for these changes, the following may be of significance:

In the batch tests, in which responses under aerobic conditions following anaerobic acetate addition were used to determine values for $\hat{\mu}_{G_1}$ and $f_{P,upt}$ [see section 6.3(1) and (2)] the mixed liquor first was aerated for 24 hours before commencing the anaerobic acetate addition. The initial 24 hour aeration period may have affected the estimation of both $\hat{\mu}_{G_1}$ and $f_{P,upt}$. The long aeration period was applied because in the aerobic digestion tests (see Chapter 7, section 4.1) it was observed (and correctly simulated) that endogenous mass loss did not become dominant until after about 18 hours. It is possible that, by inducing a completely endogenous condition in the batch test for determining $\hat{\mu}_{G_1}$ and $f_{P,upt}$, the organism's response thereafter with substrate addition, may deviate, for a period, from the 'normal'.

Taking due account of the remarks above, the responses of the following enhanced culture systems were simulated; modified Bardenpho systems at sludge ages of 20, 10 and 7,5 days (see Figs 7.1(a) and 7.1(c) for systems setups and Tables 7.2 and 7.3 for systems responses); UCT system at a sludge age of 10 days (see Fig 7.1(b) for system setup and Table 7.2 for system response). In Tables 8.5 to 8.8 the observed

Table 8.5: Measured and predicted responses for 20 day sludge age modified Bardenpho enhanced culture system.

Parameter	Sample Point	Value	
		Measured	Predicted
COD (mgCOD/l)	Influent	544,0	462,3*
	Effluent	62,0	62,6
TKN (mgN/l)	Influent	35,2	35,2
	Effluent	2,5	1,8
VSS (mgVSS/l)	Reactor 5	2998	3075
Carbonaceous OUR (mgO/l/h)	Reactor 3	19,9	20,0
	Reactor 4	15,3	14,8
	Reactor 5	10,3	8,2
	Underflow aeration	14,5	12,7
P(mgP/l)	Influent	53,6	53,6
	Anaerobic 1	145,8	147,8
	Anoxic 2	76,8	80,1
	Aerobic 3	49,1	58,7
	Aerobic 4	23,4	31,6
	Aerobic 5	5,6	6,2
	Underflow aeration	22,2	0,2
P removal (mgP/l)		49,7	47,6

*"Predicted" influent COD = (COD measured-COD denitrified).

"Predicted" influent COD includes unbiodegradable soluble COD due to the yeast extract, equal to 25 mgCOD/l.

Table 8.6: Measured and predicted responses for 10 day sludge age modified Bardenpho enhanced culture system.

Parameter	Sample Point	Value	
		Measured	Predicted
COD (mgCOD/l)	Influent	417	350*
	Effluent	62,0	62,5
TKN (mgN/l)	Influent	26,9	26,9
	Effluent	2,5	1,9
VSS (mgVSS/l)	Reactor 5	1167	1276
Carbonaceous OUR (mgO/l/h)	Reactor 4	16,6	15,5
	Reactor 5	9,4	9,6
P (mgP/l)	Influent	43,8	43,8
	Anaerobic 1	115,0	120,0
	Anaerobic 2	134,0	124,4
	Anoxic 3	72,9	82,3
	Aerobic 4	38,5	49,1
	Aerobic 5	7,6	4,1
	Effluent	5,2	4,1
P removal (mgP/l)		38,6	39,7

*"Predicted" influent COD = (COD measured-COD denitrified).

"Predicted" influent COD includes unbiodegradable soluble COD due to the yeast extract, equal to 35 mgCOD/l.

Table 8.7: Measured and predicted responses for 7,5 day sludge age modified Bardenpho enhanced culture system.

Parameter	Sample Point	Value	
		Measured	Predicted
COD (mgCOD/l)	Influent	410	350*
	Effluent	53,0	56,3
TKN (mgN/l)	Influent	26,3	26,3
	Effluent	2,5	3,8
VSS (mgVSS/l)	Reactor 5	1036	996
Carbonaceous OUR (mgO/l/h)	Reactor 4	15,2	14,7
	Reactor 5	7,8	9,6
P (mgP/l)	Influent	46,0	46,0
	Anaerobic 1	102,12	119,0
	Anaerobic 2	132,14	122,6
	Anoxic 3	72,6	80,8
	Aerobic 4	38,16	49,5
	Aerobic 5	8,2	2,0
	Effluent	3,4	2,0
P removal (mgP/l)		42,64	44,0

*"Predicted" influent COD = (COD measured - COD denitrified).

"Predicted" influent COD includes unbiodegradable soluble COD due to the yeast extract, equal to 35 mgCOD/l.

Table 8.8: Measured and predicted responses for 10 day sludge age UCT enhanced culture system.

Parameter	Sample Point	Value	
		Measured	Predicted
COD (mgCOD/l)	Influent	543	450*
	Effluent	65	48,5
TKN (mgN/l)	Influent	36,0	36,0
	Effluent	2,5	1,3
VSS (mgVSS/l)	Reactor 5	2397	2260
Carbonaceous OUR (mgO/l/h)	Reactor 3	15,4	20,7
	Reactor 4	14,1	15,9
	Reactor 5	10,9	8,9
P (mgP/l)	Influent	63,7	63,7
	Anaerobic 1	157,5	157,2
	Anoxic 2	86,3	90,0
	Aerobic 3	48,2	60,5
	Aerobic 4	12,7	28,7
	Aerobic 5	4,8	1,5
	Effluent	2,9	1,5
P removal (mgP/l)		60,94	62,2

**Predicted" influent COD = (COD measured-COD denitrified).

"Predicted" influent COD includes unbiodegradable soluble COD due to the yeast extract, equal to 25 mgCOD/l.

and predicted responses for the respective systems are listed. Comparing the observed and predicted responses it is evident that:

- (1) Over the range of sludge ages, from 7,5 to 20 days, using *one* set of constants the model appears to predict the behaviour closely.
- (2) Except for one reactor, no discernable inconsistency is apparent, nor any deviatory tendency with sludge age. The exception is the underflow aeration reactor in the 20 day sludge age modified Bardenpho system (Table 8.5) where there is an observed P concentration of 22 mgP/l as against the simulated 0,2 mgP/l.

7.3 Conclusion

When due account is taken of the complexity of the biological excess P removal phenomenon, the behaviour predicted by the model conforms surprisingly well to the behaviour observed in the experimental batch and steady state system studies. The closeness with which the predictions conform to the observations over the wide range of conditions constitutes evidence for the acceptability of the model.

8. CONCLUSIONS

The mathematical model presented in this paper deals specifically with enhanced cultures of polyP organisms receiving acetate only as substrate. However the process for conversion of readily biodegradable substrate to 'acetate-like' substrates is included in anticipation of the model being developed further to be applied to situations where sewage serves as substrate. The mathematical model deals only with the anaerobic and aerobic states; anoxic states are not dealt with because the experimental enhanced cultures developed only induce minimal growth of polyP organisms that utilize nitrate as electron acceptor. However, this aspect requires further investigation, because in systems receiving municipal sewages, on occasion denitrification and P uptake have been observed at laboratory and full-scale.

Despite the restrictions discussed above, the model constitutes a major step towards incorporating biological excess P removal in the general activated sludge model. This incorporation may yet turn out to be less difficult than a subjective assessment would lead one to presume, for the following reasons: From the experimental investigation it would appear that the polyP and the non-polyP populations act relatively independently. Independence of action arises principally from the apparent non-predation of polyP organisms, whereas non-polyP ones suffer heavy predation.

The principal sources of interaction appear to be;

- (1) in the anaerobic phase where the short chain fatty acids generated by the non-polyP organisms are sequestered by the polyP organisms and,
- (2) in the endogenous mass loss of the polyP organisms where soluble endogenous COD generated can be utilized by the non-polyP organisms.

Competition between the population groups for the same substrate although it influences the solution, does not influence the structure of the joint model, because this will be governed by kinetics not by interaction.

9. REFERENCES

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CHAPTER 9

THE FUTURE

1. INTRODUCTION

Having presented the results of this investigation, it is perhaps useful to set down briefly the present status of the theory on the biological excess P removal phenomenon in nutrient removal activated sludge systems, the implications of the theory on full-scale system behaviour, design and operation, the deficiencies in the basic theory, the expected orientation in future research and, the ancillary problems that have emerged in the application of biological excess P removal technology at full-scale.

2. BIOLOGICAL EXCESS P REMOVAL THEORY

2.1 Present status

With regard to the understanding of the biological excess P removal phenomenon, the following appear to have been established:

- (1) The principal microorganisms mediating biological excess P removal in activated sludge systems (polyP organisms), belonging to the genus *Acinetobacter*.
- (2) Species from this genus are present in significant concentration in most activated sludge systems, in completely aerobic, anoxic/aerobic and anaerobic/anoxic/aerobic systems.
- (3) Characteristics of these species, of specific importance to biological excess P removal are:
 - (i) They do not possess an Embden-Meyerhof pathway; some strains possess an Entner-Doudoroff pathway. This latter pathway is inoperative under anaerobic conditions. As a consequence these organisms cannot break down "glucose-like" materials to short chain fatty acids under anaerobic conditions. Under aerobic conditions, however, those strains with an Entner-Doudoroff pathway can utilize "glucose-like" materials and because of this can compete successfully with other organisms in activated sludge systems, which explains their presence in completely aerobic systems.

- (ii) They can store short chain fatty acids (e.g. acetate) as poly- β -hydroxybutyrate (PHB) and phosphorus as polyphosphate (polyP), but these propensities are invoked only under appropriate sets of environmental conditions. In biological excess P removal systems, the optimal appropriate set is the presence of short chain fatty acids in an anaerobic environment, for PHB storage by cleavage of polyP, followed by an aerobic environment, for storage of polyP by utilizing some of the PHB.
- (4) Normal municipal wastewaters usually contain very little or no short chain fatty acids. If readily biodegradable ("glucose-like") COD is present in the anaerobic zone, this COD fraction is converted to short chain fatty acids by the non-polyP facultative anaerobes, thereby providing substrate to the polyP organisms for PHB storage. A kinetic model describing this conversion, with the associated P release, is available.
- (5) A biochemical model is available which describes the pathways for polyP and PHB synthesis and degradation, and the control mechanisms governing these pathways. The model explains the behaviour of *Acinetobacter* spp. over a wide range of imposed environmental conditions, such as under anaerobic, anoxic and aerobic conditions with glucose, acetate and PHB present, or no substrate present.
- (6) Procedures are available to develop enhanced cultures of polyP organisms in modified Bardenpho, UCT and Phoredox/AO systems using acetate as influent substrate, with mineral nutrient and growth factor additions. Using these procedures, enhanced cultures have been developed in modified Bardenpho and UCT systems in which the polyP organisms, *Acinetobacter* spp., constitute 90 percent or more of the total organism mass. In these enhanced culture systems the behaviour of the polyP organisms dominate the system response.
- (7) The enhanced cultures have provided the base material for experimental investigations which, under different imposed test conditions, has allowed identification of the processes and compounds involved in biological excess P removal. These in turn have provided the basis for setting up a mechanistic model which provides a qualitative macroscopic description of the biological excess P removal phenomenon.

- (8) The experimental results also have provided information on the form of the kinetics of the processes, and their stoichiometric interactions with the compounds. From this information, using the mechanistic model as a basis, the process rates and stoichiometry have been formulated mathematically and incorporated into a kinetic model for biological excess P removal in enhanced cultures.
- (9) Evidence to date has verified that the kinetic model describes the response of enhanced cultures with adequate accuracy and precision over ranges of imposed environmental conditions, substrate addition and steady state system operation.

2.2 Implications on system behaviour, design and operation

Having a fairly clear understanding of the biochemistry/kinetics of the biological excess P removal phenomenon assists both the designer and the operator of biological excess P removal plants. It,

- (1) explains the need for anaerobic/aerobic sequencing, P release and uptake observed in the anaerobic and aerobic reactors respectively, and the occurrence of secondary release;
- (2) explains the deleterious influence of nitrate discharge to the anaerobic reactor;
- (3) provides guidelines for sizing the anaerobic reactor and,
- (4) indicates the advantage of subdividing the anaerobic reactor (and the aerobic reactor) into two or more reactors in series;
- (5) provides a guideline on the influence of sludge age on biological excess P removal;
- (6) establishes the importance of the influent total COD and the influent readily biodegradable COD fraction for biological excess P removal and,
- (7) points to procedures for improving P removal by, for example, augmenting the readily biodegradable COD by acid fermentation of primary sludge.

2.3 Deficiencies

Although the biochemical model provides guidance on expected behaviour of the polyP organisms under *anoxic* conditions, it has not been possible to develop a mechanistic/kinetic model for anoxic behaviour. The reason is that, in the enhanced cultures, the denitrification by the polyP organisms is relatively minor and information obtained from the results is inadequate to allow identification of the processes, their rates and stoichiometry. Probably the main area of ignorance is in kinetics of the anoxic behaviour of *Acinetobacter* spp.

2.4 Future work

The kinetic model for biological excess P removal is specific to enhanced cultures of polyP organisms. This model needs to be incorporated into the general activated sludge model. The indications are that the non-polyP and polyP population groups act relatively independently of each other so that integration of the polyP organism kinetic model with the general activated sludge kinetic model should not present undue difficulties. This independence of action also suggests that development of a model for design purposes should not be inordinately difficult. However, certain aspects in the general model itself will require attention for realistic integration of the polyP organism model: In the present general model, for nitrification/denitrification activated sludge systems, in the primary anoxic zone denitrification takes place using readily biodegradable and slowly biodegradable COD. In the integrated model, the readily biodegradable COD will be stored in the anaerobic zone and, noting the poor denitrification performance of the polyP organism mass, the readily biodegradable COD fraction no longer will contribute to denitrification in the primary anoxic zone. Yet, in biological excess P removal systems (which always include an anaerobic reactor) the denitrification in the primary anoxic zone remains high. It would appear that, in the anaerobic zone, not only is the readily biodegradable COD fraction converted to short chain fatty acids (for sequestration by the polyP organisms), but the slowly biodegradable COD fraction also is modified in some fashion so that the specific denitrification rate associated with the slowly biodegradable COD increases significantly over the specific rate when there is no preceding anaerobic reactor. As the primary anoxic reactor has a significant influence on the denitrification capacity of the system, there is a need to identify the mechanisms in the anaerobic zone that give rise to the modification of the slowly biodegradable COD and to incorporate these effects into the integrated activated sludge model.

3. ANCILLARY PROBLEMS

Operation of full-scale biological excess P removal plants has brought to the fore a number of problems which have implication on the design and operation of these systems.

3.1 Bulking

Nitrification/denitrification/P removal plants usually are operated at sludge ages from 10 to 25 days. At these long sludge ages the plants almost invariably produce bulking sludges (with Diluted Sludge Volume Indices ranging from 120-350 ml/g). The filaments present characteristically include *Microthrix parvicella* and Type 0092; these sort under the so-called low F/M or long sludge age filaments. Proliferation of these filaments can be controlled by chlorination but this is an expensive procedure and addresses the symptom, not the cause.

Bulking sludges have a significant impact on the economics and design of nutrient removal plants; elucidation of the causes for bulking therefore merits a major research effort, to develop specific methods for controlling the proliferation of the filaments.

3.2 Sludge treatment and disposal

Waste activated sludge from biological excess P removal systems contains high concentrations of stored P. If the sludge turns anaerobic some of the P will be released. This often is observed during gravity thickening of the waste sludge prior to anaerobic digestion. If the supernatant is returned untreated to the head of the activated sludge system then the nett P removal by the system is reduced. Thus, either the supernatant must be treated chemically to precipitate the P and the resultant precipitant removed from the return flow or thickening should be done by, say, dissolved air flotation which keeps the sludge in an aerobic state.

The waste sludge from biological excess P removal systems also contains relatively high concentrations of stored magnesium (Mg) which stabilizes the polyP. When the waste sludge is anaerobically digested, the P and Mg are released and can combine with free and saline ammonia to give rise to precipitation of struvite. This precipitant can cause blockages in pipes conveying the treated sludge. The factors influencing the precipitation have not yet been clearly delineated so that effective countermeasures cannot be developed.

3.3 Chemical backup systems

For all biological excess P removal systems it is desirable that chemical backup is provided. All biological systems at times exhibit reduced efficiency. Chemical backup increases the reliability of the system and creates confidence in the user.

Any breakdown in the aeration provision of biological excess P removal systems will create temporary anoxic or anaerobic states, and lead to massive release of P from the polyP organisms equal to several days of influent P mass. Again chemical backup is desirable to limit the P discharge during this crisis period. Control procedures for addition of chemicals, and the types of chemicals for optimal control (and in South Africa minimum increase in dissolved salts) still require research attention.

3.4 Short chain fatty acid augmentation

In a correctly designed biological excess P removal system, the concentration of P that can be removed is almost directly proportional to the influent readily biodegradable COD concentration. With some wastewaters this readily biodegradable COD concentration is inadequate for the required P removal. Research on full-scale systems has indicated that the readily biodegradable COD fraction of the influent can be augmented by acid fermentation of the underflow from the primary settling tanks and returning either the supernatant (from the fermented sludge), or the fermented mixed liquor itself, to the influent feed line of the activated sludge system. Identification of the optimal conditions for acid fermentation is still not possible and merits further research.

4. CLOSURE

The fact of biological excess P removal is now firmly established and the mechanisms sufficiently understood. Research into P removal *per se* will provide greater clarity, but technically there is adequate understanding to exploit the phenomenon at full scale. Essentially the future of biological excess P removal no longer depends on any better understanding of the phenomenon, but on a better understanding of the problems that can develop in the operation of these plants, i.e. control of filamentous organism growth; augmentation of the readily biodegradable COD fraction; efficient backup systems to maintain high P removal efficiency and to cope with massive P discharges through system malfunctions and; procedures to limit P feedback to the system arising from the liquid phase in sludge digestion and sludge drying.

APPENDIX A

SYMBOL SYSTEM

At the University of Cape Town (UCT) the first activated sludge model (a steady state model) was developed in 1969. Thereafter a series of models were developed, sequentially incorporating nitrification, denitrification, bisubstrate concept and the death-regeneration hypothesis. With each extension, the symbols previously defined had to be critically re-examined for consistency and for easy recognition. The most severe test for the symbolization has been to make provision also for biological excess phosphorus (P) removal processes and compounds in the general scheme for symbolization.

With the incorporation of biological excess (P) removal some new symbols had to be developed and, for consistency, some of the previous symbols had to be modified. The total list of symbols, and what they represent, is given in Table A.1.

Also given in Table A.1 are the equivalent symbols recommended by the IAWPRC task group. The reader will note that the symbols proposed by the UCT group are not the same as those recommended by the IAWPRC task group - the basis for naming the symbols differs sharply between the two symbol systems:

In the UCT symbol system, subdivision of the compounds is on the basis of substrate (S), volatile mass (X-VSS units or Z-COD units), nitrogen (N), phosphorus (P) and oxygen (O). In the IAWPRC symbol system, subdivision of the compounds is on the basis of particulate (X) or soluble (S).

Irrespective of the merits of the two symbol systems, the UCT symbol system has been retained to accommodate those who have achieved familiarity with this system from reading past papers.

Table A.1: General UCT symbol system, updated to include biological excess phosphorus removal, with IAWPRC symbol equivalent.

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
MAIN SYMBOLS		
S	Substrate	X or S
X	Volatile solids in VSS units	X
Z	Volatile solids in COD units	X
N	Nitrogen	X or S
P	Phosphorus	X or S
O	Oxygen	S _O
f	Fractional contents	f
SUBSCRIPTS		
B	Biological (active) mass	B
E	Endogenous mass	E
I	Inert mass	I
H	Heterotrophs	H
A	Autotrophs (nitrifiers)	A
G	PolyP organisms	—
b	Biodegradable	—
u	Unbiodegradable	—
p	Slowly biodegradable (particulate)	—
s	Readily biodegradable (soluble)	—
i	Influent	—
e	Effluent	—
t	Total	—

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
COMPOUNDS		
<u>S</u>	<u>Substrate</u>	
S_u	Unbiodegradable	—
S_{up}	Unbiodegradable, particulate	X_I
S_{us}	Unbiodegradable, soluble	S_I
S_b	Biodegradable	—
S_{bs}	Biodegradable, readily (soluble)	S_S
$S_{bs,a}$	Biodegradable, readily (soluble), acetate	—
$S_{bs,c}$	Biodegradable, readily (soluble), complex	—
S_{bp}	Biodegradable, slowly (particulate)	X_S
S_{phb}	Stored PHB	—
S_{abs}	Absorbed	—
S_{enm}	Enmeshed	X_S
<u>Z</u>	<u>Volatile solids (COD units)*</u>	
Z_B	Biological (active) mass	
$Z_{B,H}$	Biological (active) mass, Heterotrophs	$X_{B,H}$
$Z_{B,A}$	Biological (active) mass, Autotrophs	$X_{B,A}$
$Z_{B,G}$	Biological (active) mass, PolyP organisms	—
Z_E	Endogenous mass	X_E
$Z_{E,H}$	Endogenous mass, Heterotrophs	$X_{E,H}$
$Z_{E,A}$	Endogenous mass, Autotrophs	$X_{E,A}$
$Z_{E,G}$	Endogenous mass, PolyP organisms	—
Z_I	Inert mass	X_I
Z_V	Total volatile solids (COD units)	X_V
	$= Z_B + Z_E + Z_I$	
	$= X_v \cdot f_{cv}$	

*Following the IAWPRC task group proposals volatile solids are expressed in COD units. The symbol X is substituted for Z if VSS units are used.

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
<u>N</u>	<u>Nitrogen</u>	
N _o	Organic	—
N _{o,u}	Organic, unbiodegradable	—
N _{o,up}	Organic, unbiodegradable, particulate	X _{NI}
N _{o,us}	Organic, unbiodegradable, soluble	—
N _{o,bp}	Organic, biodegradable	—
N _{o,bs}	Organic, biodegradable, soluble	S _{ND}
N _{h3}	Ammonia	S _{NH}
N _{o3}	Nitrate	S _{NO}
N _Z	Nitrogen in volatile solids	—
N _{ZB}	Nitrogen in volatile solids, biological (active) mass	—
N _{ZB,H}	Nitrogen in volatile solids, biological (active) mass, heterotrophs	—
N _{ZB,A}	Nitrogen in volatile solids, biological (active) mass, autotrophs	—
N _{ZB,G}	Nitrogen in volatile solids, biological (active) mass, polyP organisms	—
N _{ZE}	Nitrogen in volatile solids, endogenous mass	—
N _{ZE,H}	Nitrogen in volatile solids, endogenous mass, heterotrophs	—
N _{ZE,A}	Nitrogen in volatile solids, endogenous mass, autotrophs	—
N _{ZE,G}	Nitrogen in volatile solids, endogenous mass, polyP organisms	—
N _{ZI}	Nitrogen in volatile solids, inert mass	—

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
P	<u>Phosphorus</u>	
P_P	Particulate	X_P
P_S	Soluble	S_P
P_Z	Phosphorus in volatile solids	—
P_{ZB}	Phosphorus in volatile solids, biological (active) mass	—
$P_{ZB,H}$	Phosphorus in volatile solids, biological (active) mass, heterotrophs	—
$P_{ZB,A}$	Phosphorus in volatile solids, biological (active) mass, autotrophs	—
$P_{ZB,G}$	Phosphorus in volatile solids, biological (active) mass, polyP organisms	—
P_{ZE}	Phosphorus in volatile solids, endogenous mass	—
$P_{ZE,H}$	Phosphorus in volatile solids, endogenous mass, heterotrophs	—
$P_{ZE,A}$	Phosphorus in volatile solids, endogenous mass, autotrophs	—
$P_{ZE,G}$	Phosphorus in volatile solids, endogenous mass, polyP organisms	—
P_{ZI}	Phosphorus in volatile solids, inert mass	—
P_{polyP}	Stored polyphosphate	—

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
f	FRACTIONAL CONTENTS	f**
f_S	<u>Substrate</u> (fraction of substrate that is...)	—
f _{S,u}	unbiodegradable	—
f _{S,us}	unbiodegradable, soluble	—
f _{S,up}	unbiodegradable, particulate	—
f _{S,b}	biodegradable	—
f _{S,bs}	biodegradable, readily (soluble)	—
f _{S,bsa}	biodegradable, readily (soluble), acetate	—
f _{S,bsc}	biodegradable, readily (soluble), complex	—
f _{S,bp}	biodegradable, slowly (particulate)	—
f_Z	<u>Volatile solids (COD units)</u> (fraction of volatile solids that is...)	
f _{Z,B}	biological (active) mass	—
f _{Z,BH}	biological (active) mass, heterotrophs	—
f _{Z,BA}	biological (active) mass, autotrophs	—
f _{Z,BG}	biological (active) mass, polyP organisms	—
f _{Z,E}	endogenous mass	—
f _{Z,EH}	endogenous mass, heterotrophs	—
f _{Z,EA}	endogenous mass, autotrophs	—
f _{Z,EG}	endogenous mass, polyP organisms	—
f _{Z,I}	inert mass	—

** No information is available in the literature on the subscripts for fraction contents, using the IAWPRC symbol system.

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
f_N	<u>Nitrogen</u> (fraction of nitrogen that is...)	
$f_{N,o}$	organic	—
$f_{N,ou}$	organic, unbiodegradable	—
$f_{N,ous}$	organic, unbiodegradable, soluble	—
$f_{N,oup}$	organic, unbiodegradable, particulate	—
$f_{N,ob}$	organic, biodegradable	—
$f_{N,obs}$	organic, biodegradable, soluble	—
$f_{N,obp}$	organic, biodegradable, particulate	—
$f_{N,h3}$	ammonia	—
f_P	<u>Phosphorus</u> (fraction of phosphorus that is...)	
$f_{P,p}$	particulate	—
$f_{P,s}$	soluble	—

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
$f_{,N}$	<u>Nitrogen content</u> (fraction of 'compound' that is nitrogen)	
$f_{Z,N}$	Volatile solids (COD units)	—
$f_{ZB,N}$	Volatile solids (COD units), biological (active) mass	i_{XBN}
$f_{ZBH,N}$	Volatile solids (COD units), biological (active) mass, heterotrophs	—
$f_{ZBA,N}$	Volatile solids (COD units), biological (active) mass, autotrophs	—
$f_{ZBG,N}$	Volatile solids (COD units), biological (active) mass, polyP organisms	—
$f_{ZE,N}$	Volatile solids (COD units) endogenous mass	i_{XEN}
$f_{ZEH,N}$	Volatile solids (COD units), endogenous mass, heterotrophs	—
$f_{ZEA,N}$	Volatile solids (COD units), endogenous mass, autotrophs	—
$f_{ZEG,N}$	Volatile solids (COD units), endogenous mass, polyP organisms	—
$f_{ZI,N}$	Volatile solids (COD units), inert mass	—

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
$f_{\underline{P}}$	<u>Phosphorus content</u> (fraction of "compound" that is phosphorus)	
$f_{Z,P}$	Volatile solids (COD units)	—
$f_{ZB,P}$	Volatile solids (COD units), biological (active) mass	i_{XBP}
$f_{ZBH,P}$	Volatile solids (COD units), biological (active) mass, heterotrophs	—
$f_{ZBA,P}$	Volatile solids (COD units), biological (active) mass, autotrophs	—
$f_{ZBG,P}$	Volatile solids (COD units), biological (active) mass, polyP organisms	—
$f_{ZE,P}$	Volatile solids (COD units) endogenous mass	i_{XEP}
$f_{ZEH,P}$	Volatile solids (COD units), endogenous mass, heterotrophs	—
$f_{ZEA,P}$	Volatile solids (COD units), endogenous mass, autotrophs	—
$f_{ZEG,P}$	Volatile solids (COD units), endogenous mass, polyP organisms	—
$f_{ZI,P}$	Volatile solids (COD units), inert mass	—

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
f_E	<u>Endogenous residue</u> (fraction of the organism that remains as unbiodegradable residue)	
f_{Ep}	unbiodegradable, particulate residue	f_E
$f_{Ep,H}$	unbiodegradable, particulate residue, heterotrophs	—
$f_{Ep,A}$	unbiodegradable, particulate residue, autotrophs	—
$f_{Ep,G}$	unbiodegradable, particulate residue, polyP organisms	—
f_{Es}	unbiodegradable, soluble residue	—
$f_{Es,H}$	unbiodegradable, soluble residue, heterotrophs	—
$f_{Es,A}$	unbiodegradable, soluble residue, autotrophs	—
$f_{Es,G}$	unbiodegradable, soluble residue, polyP organisms	—
	<u>Special fractional contents</u>	
f_{cv}	COD/VSS ratio	
f_i	MLVSS/MLSS ratio	
f_{NS}	Influent TKN/COD concentration ratio	

Table A.1: (Continued)

UCT SYMBOL SYSTEM		IAWPRC
Symbol	Description	Equivalent
CONSTANTS***		
<u>Y</u>	<u>True specific yield</u>	Y
Y_H	Heterotrophs	Y_H
Y_A	Autotrophs	Y_A
Y_G	PolyP organisms	—
<u>b</u>	<u>Specific endogenous mass loss rate</u>	b
b_H	Heterotrophs	b_H
b_A	Autotrophs	b_A
b_G	PolyP organisms	—
$\hat{\mu}$	<u>Maximum specific growth rate</u>	μ_{\max}
$\hat{\mu}_H$	Heterotrophs	$\hat{\mu}_H$
$\hat{\mu}_A$	Autotrophs	$\hat{\mu}_A$
$\hat{\mu}_G$	PolyP organisms	—
K_s	<u>Monod half saturation coefficient</u>	K_S
K_{sH}	Heterotrophs	K_{SH}
K_{sA}	Autotrophs	K_{SA}
K_{sG}	PolyP organisms	—
R_h	Hydraulic retention time	θ
R_s	Sludge age - solids retention time	θ_c
Q	Flow rate	Q
V	Volume	V

*** Only constants common to all three bacterial populations are given. A number of constants exist specific to each population. Constants used in this thesis are given in the list of symbols, and in the relevant chapters.

APPENDIX B

UCT SYSTEM ANAEROBIC REACTOR - MIXED LIQUOR DILUTION EFFECT

In the UCT system (see Fig B.1) the concentrations of Mixed Liquor Suspended Solids (MLSS) or Volatile Suspended Solids (MLVSS) in the anaerobic reactor(s) differ from those in the anoxic and aerobic reactors because the mixed liquor recycled from the anoxic reactor to the anaerobic reactor (via the r -recycle) is diluted by the influent waste flow. From a mass balance on the anaerobic reactor:

$$X_{ta} = X_{tb} r / (1+r) \quad (B.1)$$

where X_{ta} = mixed liquor concentration in anaerobic reactor(s)
 X_{tb} = mixed liquor concentration in anoxic/aerobic reactors
 r = (r -recycle flow rate)/(influent flow rate)

The volume fractions of the different reactors can be determined from the mass fractions as follows:

If the anaerobic mixed liquor mass fraction is f_{xa} , then the remaining mixed liquor mass fraction (in the anoxic and aerobic reactors) is $(1-f_{xa})$. The volume of the anoxic/aerobic section, V_a , is given by:

$$V_a = (1-f_{xa}) M(X_t) / X_{tb} \quad (B.2)$$

where $M(X_t)$ = mass of sludge in the system.

The volume of the anaerobic section, V_{an} , is:

$$V_{an} = f_{xa} M(X_t) / X_{ta} \quad (B.3)$$

where X_{ba} is given by Eq (B.1).

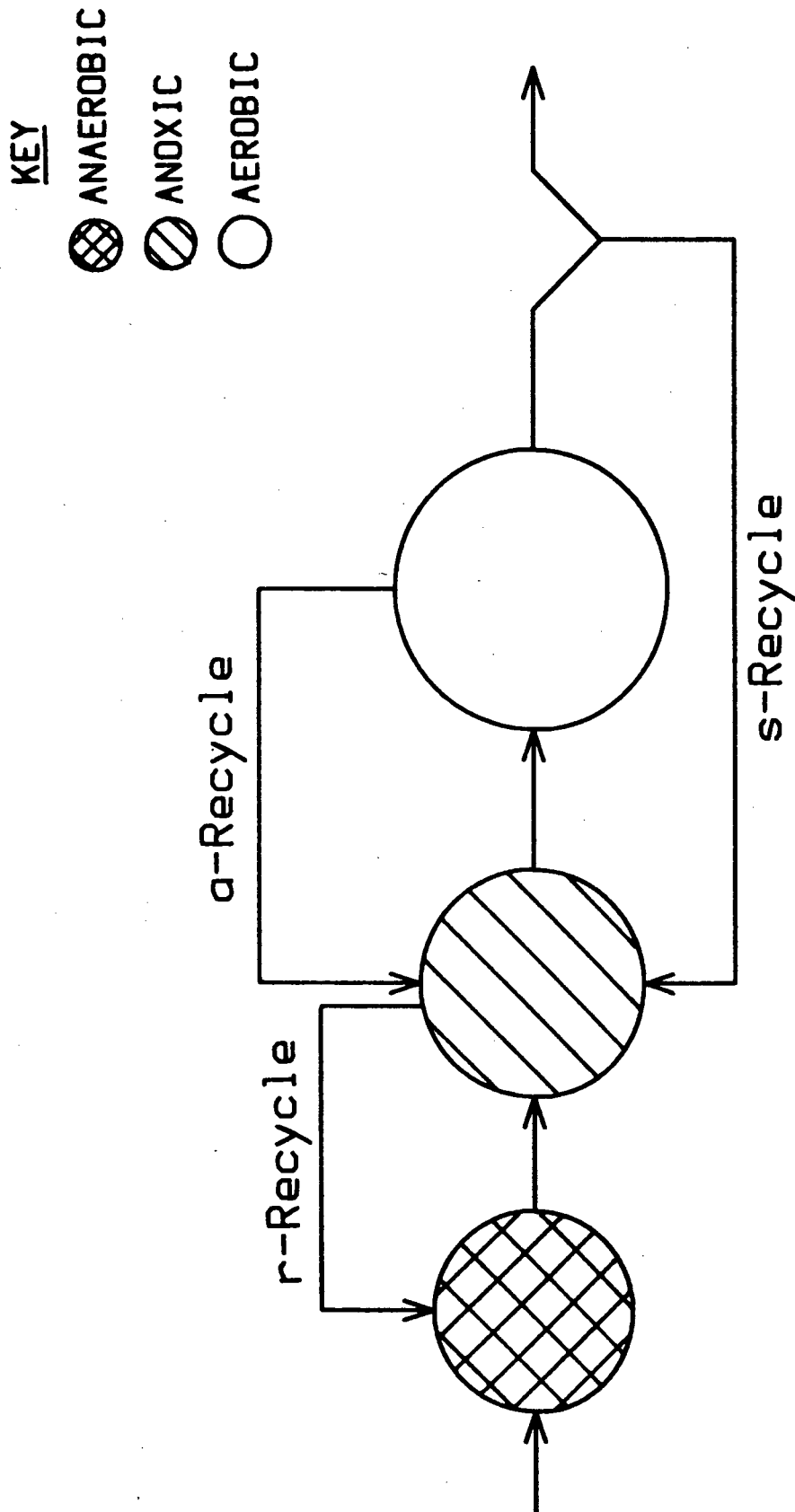


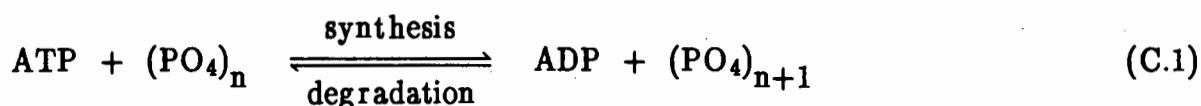
Fig B.1: Schematic layout of UCT system.

APPENDIX C

THERMODYNAMIC CONTROL OF POLYP METABOLISM

From Chapter 5, polyP metabolism can follow two pathways namely a synthesis pathway leading to phosphate uptake and a degradation pathway leading to phosphate release.

The basic equation governing both these pathways is the reversible reaction:



From an energetic point of view the direction of the reaction under non-equilibrium isothermal conditions is determined by the value of the Gibb's free energy change for the reaction (ΔG_R); a negative value for ΔG_R effecting the forward reaction and a positive value the reverse. It is of fundamental interest to delineate thermodynamic conditions favouring the forward and reverse reactions. This can be done from an analysis of the equation for ΔG_R , as follows:

The equation for the free energy change (ΔG_R) of the forward reaction (C.1), i.e. the synthesis reaction, is

$$\Delta G_R = \Delta G_R^\Theta + RT \ln \frac{(\text{PO}_4)_{n+1} \cdot (\text{ADP})}{(\text{PO}_4)_n \cdot (\text{ATP})} \quad (\text{C.2})$$

where (X) = activity of species X

R = universal gas constant

T = temperature (K)

ΔG_R^Θ = standard free energy change for the equilibrium reaction

$$= \Delta G_f^\Theta (\text{products}) - \Delta G_f^\Theta (\text{reactants}) \quad (\text{C.3})$$

ΔG_f^Θ = standard free energy of formation of one mole of the substance under standard conditions.

Referring to Eq (C.2) the polyP species, $(\text{PO}_4)_n$ and $(\text{PO}_4)_{n+1}$, are solids and their activities are therefore unity; the equation for ΔG_R therefore reduces to:

$$\Delta G_R = \Delta G_R^\Theta + RT \ln \left(\frac{\text{ADP}}{\text{ATP}} \right) \quad (\text{C.4})$$

The value for ΔG_R^Θ in Eq (C.3) is determined as

$$\Delta G_R^\Theta = \Delta G_R^\Theta(\text{PO}_4)_{n+1} + \Delta G_R^\Theta(\text{ADP}) - \Delta G_R^\Theta(\text{PO}_4)_n - \Delta G_R^\Theta(\text{ATP}) \quad (\text{C.5})$$

Noting that the phosphoryl bond between ADP and P and between $(\text{PO}_4)_n$ and $(\text{PO}_4)_{n+1}$ are identical, the standard free energy changes for hydrolysis and condensation will be closely equal i.e. ΔG_f^Θ for (ATP-ADP) will closely equal ΔG_f^Θ for $[(\text{PO}_4)_{n+1} - (\text{PO}_4)_n]$. Thus ΔG_R^Θ (in Eq. C.5) closely equals zero and Eq (C.4) reduces to

$$\Delta G_R = RT \ln \left(\frac{\text{ADP}}{\text{ATP}} \right) \quad (\text{C.6})$$

This equation forms the basis for linking polyP formation or degradation to the intracellular ATP/ADP ratio. If $\text{ATP} > \text{ADP}$ then ΔG_R will be negative and the forward reaction (i.e. polyP synthesis) is favoured. This situation arises in an aerobic state where organic substrate is not limiting. Conversely if $\text{ATP} < \text{ADP}$ then ΔG_R will be positive favouring the reverse reaction i.e. polyP degradation and concomitant ATP formation. The ATP thus formed is used for two purposes; for cell function and PHB synthesis, thereby maintaining $\text{ATP} < \text{ADP}$. This situation arises when an obligate aerobic polyP organism, with an intracellular ATP demand, is placed in an anaerobic environment.

The key rôle of the ATP/ADP ratio in thermodynamic control of polyP metabolism concurs with observed regulation of the enzyme mediating the reaction (C.2) (cf. section on polyP metabolism in Chapter 5).

APPENDIX D

PROCEDURES FOR DEVELOPMENT OF ENHANCED CULTURE OF POLYP ORGANISMS

The following procedures are proposed to develop an enhanced culture of polyP organisms:

- (1) The system must incorporate anaerobic/aerobic sequencing of the mixed liquor in which the recycling of nitrate (NO_3^-) to the anaerobic zone is kept to a minimum. This can be accomplished by setting up a UCT or a 3-stage modified Bardenpho system, the 3-stage modified Bardenpho being the simplest. Systems with sludge ages of 10 and 20 days have been successfully used to obtain enhanced cultures. An anaerobic mass fraction of 30 percent (for P release) and an anoxic mass fraction of about 8 percent (for nitrate reduction) are recommended. The anaerobic mass fraction is greatly in excess of that theoretically required to sequester the acetate. Lower mass fractions (down to 10 percent) have been used successfully, but experience has shown that small fractions can, on occasion, result in 'leakage' of acetate through the anaerobic reactor; leakage appears to stimulate growth of *Pseudomonas* spp. which, once established, can lead to complete deterioration of polyP organism growth.
- (2) The aerobic zone should consist of a series of reactors, to approach plug flow, rather than a single completely mixed reactor. This will promote greater efficiency in P uptake as the uptake reaction is of a first order nature. A minimum of three aerobic reactors in series is suggested. The a-recycle to the anoxic zone should preferably be from the second aerobic reactor in the series, at around 3:1 with regard to the influent flow. With some response situations it may help to include a small aerobic reactor (± 8 percent mass fraction) in the underflow recycle (see Chapter 6).
- (3) The system may be started using mixed liquor from a municipal activated sludge system, preferably, but not necessarily, one that is exhibiting excess P removal.
- (4) Acetate has been found to be a satisfactory substrate. (This does not imply that other short chain fatty acids would be unsuitable). On starting the

system, an acetate-sewage mixture, 5:95, is suggested as influent. The system is run until all the added acetate is removed in the anaerobic zone (or until P release appears to have attained a maximum steady state value). The acetate fraction of the influent then is increased incrementally at say 5 percent increments, the sewage fraction being correspondingly decreased. Mineral nutrients and growth factors must be added to the influent in proportion to the acetate added, see Chapter 6. Sufficient P needs to be added to the influent to ensure that P is always present in the effluent, roughly 0,12 mgP/mgCOD as acetate. Addition of P as K_2HPO_4 will ensure sufficient K in the influent.

- (5) Acid must be added as a sidestream to each aerobic reactor to maintain pH $\pm 7,4$, to exclude precipitation of P and prevent inhibition of the polyP organism due to too high a pH.
- (6) Settling tank design must be adequate due to the unique settling problems of the enhanced culture. Settling tank preferentially should be of the sloping type with a recycle of about 1:1 from the underflow to the influent flow of the settler.
- (7) The reactors should be equipped with extra overflow pipes because the sludge tends to block the tubes connecting the reactors. Trays should be provided underneath the system to catch spillage; such trays should be plastic coated to prevent contact of the sludge with zinc and other metal coatings. Contact with these coatings can act very adversely on the system response.
- (8) The system volumes and influent loads must be selected such that VSS is less than 2500 mgVSS/ ℓ . The following design procedure is suggested:

$$MX_v = Q \cdot S_{ti} \left(\frac{Y \cdot R_s}{1 + b \cdot R_s} \right) (1 + f \cdot b \cdot R_s) \quad (D.1)$$

where Q = influent flow rate (ℓ/d)
 S_{ti} = influent COD concentration (mgCOD/ ℓ as acetate, propionate, butyrate, lactate)
 R_s = sludge age (d)
 MX_v = system volatile solids mass (mgVSS)

D.3

- Y = specific yield
= 0,45 (mgVSS/mgCOD) for polyP organisms
- b = specific endogenous mass loss rate
= 0,04(/d) for polyP organisms
- f = endogenous residue fraction
= 0,25 (mgVSS/mgVSS) for polyP organisms.

$$X_v = \frac{MX_v}{V} \quad (D.2)$$

$$X_t = X_v / f_i \quad (D.3)$$

- where V = volume of system for modified Bardenpho (ℓ)
- X_v = volatile solids concentration (mgVSS/ ℓ)
- X_t = total solids concentration (mgTSS/ ℓ)
- f_i = VSS/TSS
= 0,46 (mgVSS/mgTSS) for polyP organisms.

- (9) Fresh influent must be made daily and the influent kept in a covered drum at 4° C. The drum and feed line must be cleaned with boiling water daily.

APPENDIX E

MATRIX METHOD FOR MODEL PRESENTATION

To fully understand the mathematical model presented in Chapter 8, Table 8.1, it is useful to gain an insight into the representation and workings of the matrix as described below.

Representation

The matrix is represented by a number of columns and rows; one column for each compound and one row for each process. The symbols for the compounds are listed at the head of the appropriate column and the compounds are defined at the bottom of the corresponding column. The index 'i' is assigned to identify a compound in the totality of compounds.

The processes are itemized one below the other down the left-hand side of the matrix. The index 'j' is assigned to identify the process. The process rates are formulated mathematically and listed down the right-hand side of the matrix, in line with the respective process row. These process rates are given the symbol ' ρ_j ', where j identifies the process.

Along each process row the stoichiometric coefficient for conversion from one compound to another is inserted so that each column lists the processes that influence that compound. The stoichiometric coefficients are given the symbol ' $\nu_{i,j}$ ', where i denotes the index of the compound and j the index of the process. The stoichiometric coefficients ν_{ij} are greatly simplified by working in consistent units; in this case concentrations are expressed in COD, phosphorus (P) or nitrogen (N) units. Sign convention in the matrix for the stoichiometric coefficients is 'negative for consumption' and 'positive for production'.

This matrix forms a succinct summary of the complex interactions between compounds and processes. The matrix in effect constitutes a fingerprint uniquely characterizing the phenomenon. It allows alterations in processes, compounds, stoichiometry and kinetics to be readily incorporated.

The matrix representation method has two main benefits:

- (1) It allows the effect of a particular process on the compounds to be easily determined, as follows: The reader moves along a particular row, i.e. process, and multiplies the stoichiometric coefficient (ν_{ij}) by the process rate (ρ_j). This gives the reaction rate (r_{ij}) for the particular compound being affected by the single process, i.e.

$$r = \nu_{ij} \rho_u \quad (\text{E.1})$$

In representing the matrix, by adding up the reaction rates for a particular process, a mass balance must be obtained.

- (2) It allows rapid and easy recognition of the fate of each compound, as follows: The reader moves down the column representing the compound of interest, and multiplies the stoichiometric coefficient (ν_{ij}) by the process rate (ρ_j). The summation of these multiplications gives the overall reaction rate (r_i) for the compound, i.e.

$$r_i = \sum_j \nu_{ij} \rho_j \quad (\text{E.2})$$

Switching function

Under certain conditions the process rate equations are not operative, e.g. aerobic processes are not operative under anaerobic conditions. Mathematically, switching the process rate "on" and "off" can be achieved by multiplying the appropriate rate by a "switching" factor, which is zero when the process rate is inoperative, or unity when the process rate is operative. The general expression used for the switching function is:

$$\frac{C}{K + C} \quad (\text{E.3})$$

where C = concentration of compound effecting the switch
 K = constant.

This is a Monod-type expression. By selecting very small values for K , the function is close to unity when C is present. The function decreases to zero only at very low concentrations of C . A Monod-type expression is utilized as it provides continuity

between the 'off' and the 'on' situation which helps to eliminate problems of numerical instability in computer calculations. Sometimes numerous switching functions are required, for example, anaerobic processes must be inoperative when oxygen or when nitrate is present.

Matrix solution

Solution of the matrix can be fixed in time (e.g. batch test), space (e.g. steady state multiple reactor system), or time and space (e.g. multiple reactor system with time varying flow).

- (1) Solution in time: This solution requires that the initial concentration be known whereafter changes in concentration are determined by integrating forward in time. Integration forward follows the basic Euler equation or equivalent:

$$C(t + \Delta t) = C(t) + \left(\frac{dC}{dt}\right)_t \Delta t \quad (\text{E.4})$$

where C = compound concentration

t = time

Δt = step size in integration

$\left(\frac{dC}{dt}\right)_t$ = reaction rate

The reaction rate is obtained from the summation down the particular compound's column of the multiplication terms $\nu_{ij}\rho_j$, as described previously.

- (2) Solution in space: Solution of the matrix in space requires that the transport terms be included. Inclusion of the transport terms and the rate equations is facilitated by use of the mass balance equation:

$$\begin{bmatrix} \text{Mass rate} \\ \text{of} \\ \text{accumulation} \end{bmatrix} = \begin{bmatrix} \text{Mass rate} \\ \text{of} \\ \text{input} \end{bmatrix} - \begin{bmatrix} \text{Mass rate} \\ \text{of} \\ \text{output} \end{bmatrix} + \begin{bmatrix} \text{Mass rate of} \\ \text{production} \\ \text{by reaction} \end{bmatrix}$$

The mass of input and output are the transport terms and depend on the physical characteristics of the system being modelled. The mass of production for a particular compound is obtained from the matrix. Taking an example, in symbols, for completely mixed reactor:

$$V \frac{dC}{dt} \text{ out} = Q_{\text{in}} C_{\text{in}} - Q_{\text{out}} C_{\text{out}} - r_i V \quad (\text{E.5})$$

where V = Volume

Q_{in} = Flow rate in

Q_{out} = Flow rate out

C_{in} = Concentration of compound in flow

C_{out} = Concentration of compound in outflow (i.e. reactor concentration for completely mixed reactor)

$\frac{dC_{\text{out}}}{dt}$ = rate of change of reactor concentration of compound C

$r_i = \sum_j \nu_{ij} \rho_j$, obtained from the matrix (see Eq E.2).

Dividing by V and recognizing that at steady state $\frac{dC_{\text{out}}}{dt} = 0$

$$\frac{Q_{\text{in}}}{V} C_{\text{in}} - \frac{Q_{\text{out}}}{V} C_{\text{out}} - r_i = 0$$

Mass balance equations are derived for each compound in every reactor (including the settler). This yields a set of simultaneous non-linear equations for each reactor which then may be solved to give values for all the compounds. As the equations are non-linear repetitive techniques must be employed in the solution.

- (3) Solution in time and space: This solution is confined to the situation of single or multiple reactors under repetitive diurnal flows. Again mass balances are set up but, unlike the steady state system, $(\frac{dC_{\text{out}}}{dt})$ no longer equal zero. Initial concentration values are selected and the mass balance equations are integrated forward until the solution is reached; this is achieved when the concentration of all compounds in each reactor at the start and at the end of the diurnal cycle are equal.